UNIVERSITY OF ALBERTA

Phenylbutenoic Acids as Inhibitors of Peptide Amidation

by

Kelly A. E. O'Callaghan

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

DEPARTMENT OF CHEMISTRY

Edmonton, Alberta

Fall, 1997

National Library of Canada
Acquisitions and Bibliographic Services
395 Wellington Street
Ottawa ON K1A 0N4
Canada

Bibliothèque nationale du Canada
Acquisitions et services bibliographiques
395, rue Wellington
Ottawa ON K1A 0N4
Canada

Your file Votre référence

Our file Notre référence

The author has granted a non-exclusive licence allowing the National Library of Canada to reproduce, loan, distribute or sell copies of this thesis in microform, paper or electronic formats.

L'auteur a accordé une licence non exclusive permettant à la Bibliothèque nationale du Canada de reproduire, prêter, distribuer ou vendre des copies de cette thèse sous la forme de microfiche/film, de reproduction sur papier ou sur format électronique.

The author retains ownership of the copyright in this thesis. Neither the thesis nor substantial extracts from it may be printed or otherwise reproduced without the author's permission.

L'auteur conserve la propriété du droit d'auteur qui protège cette thèse. Ni la thèse ni des extraits substantiels de celle-ci ne doivent être imprimés ou autrement reproduits sans son autorisation.

0-612-23048-1

Canada

UNIVERSITY OF ALBERTA

LIBRARY RELEASE FORM

NAME OF AUTHOR: Kelly A. E. O'Callaghan

TITLE OF THESIS: Phenylbutenoic Acids as Inhibitors of Peptide Amidation

DEGREE: Doctor of Philosophy

YEAR THIS DEGREE WAS GRANTED: 1997

Permission is hereby granted to the University of Alberta Library to reproduce single copies of this thesis and to lend or sell such copies for private, scholarly or scientific research purposes only.

The author reserves all other publication rights and all other rights in association with the copyright in the thesis, and except as hereinbefore provided, neither the thesis nor any substantial portion thereof may be printed or otherwise reproduced in any material form whatever without the author's prior written permission.

(SIGNED) KOCallaghan

PERMANENT ADDRESS:

1 Elmsley Lane

Smiths Falls, ON

Canada, K7A 4S5

DATED: Sept. 30/97

UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommended to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled Phenylbutenoic Acids as Inhibitors of Peptide Amidation by Kelly A. E. O'Callaghan in partial fulfillment of the requirements for the degree of Doctor of Philosophy

Dr. John C. Vederas (Supervisor)

Dr. Derrick Clive

Dr. Rik Tykwinski

Dr. Monica Palcic

Dr. Bruce Malcolm

Dr. Suzanne Abrams (External)

DATED: Sept. 2, 1997

In memory of my father

from whom I inherited my curiosity

and to my mother

from whom I inherited the

determination to find the answers.

ABSTRACT

Peptides bearing *C*-terminal amides are biosynthesized from precursors which have an additional glycine residue. Hydroxylation of the glycine α-carbon, in a process dependent on oxygen, copper and ascorbic acid, gives an α-hydroxyglycine intermediate **2**, which is then cleaved stereospecifically to the amide product **3** and glyoxylate **4**. *Trans*-4-phenyl-3-butenoic acid (**9**) (PBA, IC_{50} of 0.4 μM) is a time dependent, mechanism-based, irreversible inhibitor of the amidating enzyme (PAM, peptidylglycine α-amidating monooxygenase). A hybrid of PBA **9** and another mechanism-based inhibitor, D-Phe-L-Phe-D-vinylglycine (**6**), was designed which contained the unnatural amino acid (*E*)-2-amino-4-phenyl-3-butenoic acid (styrylglycine) **12**. The primary target was the tripeptide D-Phe-L-Phe-D-styrylglycine. This was synthesized by the condensation of benzaldehyde with a protected D-aspartic acid moiety, followed by oxidative decarboxylation to give the protected styrylglycine. This compound was only a weak competitive inhibitor (IC_{50} 0.4 mM) and not a mechanism-based inactivator of PAM. This result indicated that the double bond of PBA **9** was mimicking the amide functionality of the substrate peptide and not the vinyl group of **6**.

The mode of inhibition of PAM by phenylbutenoic acid was investigated using labelled analogues to determine if the inhibitors were covalently bound in the active site. Dansyl, dabsyl, fluorescein, 6-dimethylamino-2-naphthyl sulfonyl and biotin were each attached to phenylbutenoic acid *via* a 6 carbon spacer to ensure that the label would not interfere with the inhibitor binding. The dansyl (**78**) and biotin (**80**) labelled phenylbutenoic acids were mechanism-based inhibitors of PAM, but no evidence was found for covalent attachment of these compounds to the enzyme.

To provide a variety of substituted phenylbutenoic acids for structure-activity studies, three synthetic routes were investigated. The first route involved an aldol condensation of acetone with benzaldehyde, followed by a lead tetraacetate-mediated rearrangement to give the target. In the second route, substituted styrenes were oxidized

to the phenylacetaldehydes; a Wittig reaction with a stabilized reagent then gave the methyl esters of the phenylbutenoic acid. The best route involved condensation of a 3-carbon Wittig reagent with substituted benzaldehydes to form the target molecules in a one-pot reaction. A phenylbutenoic acid derivative was also synthesized with deuterium incorporation at the key methylene carbon.

ACKNOWLEDGEMENTS

I am grateful to my supervisor, Professor John C. Vederas, for his support and guidance throughout my studies. I thank all the members in our research group who have helped me tremendously thoughout the years. I am especially grateful to Dr. Mark Zabriskie, Dr. Nancy Fregeau and Dr. Joanna Harris for their help and advice, and to Dr. John McKendrick for proof-reading this manuscript. I am forever indebted to Dr. Mark Andrews for keeping this project going during my leave of absence, for all his help that allowed me to finish it, and lastly for proof-reading this document. The assistance of our collaborators, Dr. Betty Eipper's, Dr. Liang Li's and especially Dave Schriemer for running the MALDI TOF mass spectra, is acknowledged. My thanks to the spectral and analytical services for their assistance in characterizing compounds. A special thanks to Dr. Shawna MacKinnon without whose help I could not have finished this degree.

NSERC, the Alberta Heritage Foundation, the Alberta Heritage Foundation for Medical Research, the Izaak Walton Killam Scholarship and the University of Alberta are gratefully acknowledged for financial support.

TABLE OF CONTENTS

Page

INTRODUCTION.....1

RESULTS AND DISCUSSION.....10

1. Synthesis and Biological Testing of Tripeptide Inhibitors Containing D- and L-Styrylglycine.....10
2. Synthesis and Biological Testing of Labelled Inhibitors of PAM.....24
3. Substituted Phenylbutenoic Acids as Inhibitors of PAM.....50

EXPERIMENTAL.....66

REFERENCES.....164

LIST OF TABLES

Table		Page
1.	Some Peptide Amides in the Nervous and Endocrine Systems	1
2.	A Comparison of Various Isolated PAM Enzymes	3,50
3.	Structure Activity Relationships Among Inhibitors of PAM	8
4.	Yields of Diastereomeric Tripeptides **28-31** Under Different Coupling Conditions	18
5.	Approximate IC_{50} Values for PAM Inhibitors	22
6.	.....	40
7.	.....	41
8.	.....	57
9.	.....	58
10.	.....	64
11.	Protein and Activity Assays of Enzyme Isolation	160

LIST OF FIGURES

Figures		Page
1.	Copper Binding Sites	4
2.	Three Peptide Based Inhibitors	6
3.	PBA as an Inhibitor of PAM	7
4.		7
5.	Delocalization of Radical Character in the Mechanism-Based Inhibitors	11
6.	Possible Orientation of PAM Inhibitors in the Enzyme Active Site	23
7.	Design of Labelled Inhibitors	26
8.		27
9.		32
10.		35
11.		43
12.		44
13.		45
14.		46
15.		48
16.	Retrosynthetic Analysis of Synthetic Routes to PBA	52
17.		55
18.		59

LIST OF ABBREVIATIONS

$[\alpha]$	specific rotation
A_{280}	absorbance at 280 nm
Ac	acetyl
Arg	arginine
Asp	aspartic acid
Boc	*tert*-butoxycarbonyl
BOP	benzotriazol-2-yloxytris(dimethylamino)phosphoniumhexafluoro phosphate
br	broad
^{t}Bu	*tert*-butyl
BuLi	*n*-butyl lithium
Cbz	carboxybenzyl
CRH	corticotrophin releasing hormone
d	doublet
DBH	dopamine β-hydroxylase
d.e.	diastereomeric excess
DEAD	diethyl azodicarboxylate
2,6-DMANS	2-dimethylamino-6-naphthylsulfonic acid
DMAP	dimethylaminopyridine
DMF	dimethylformamide
DPPA	diphenylphosphorylazide
EDC	1-(3-dimethylaminopropyl)-3-ethylcarbodiimide
e.e.	enantiomeric excess

EEDQ	2-ethoxy1-ethoxycarbonyl-1,2-dihydroquinoline
EI	electron ionization
ELISA	enzyme linked immunosorbent assay
Enz	enzyme
Et_2O	diethyl ether
EtOAc	ethyl acetate
FAB	fast atom bombardment
FMRF	phenylalanine-methionine-arginine-phenylalanine
Gly	glycine
GnRH	gonadotrophin releasing hormone
h	hour
HHM	histidine-histidine-methionine
His	histidine
HMDS	hexamethyldisilazane
HMPA	hexamethylphosphoramide
HOAT	1-(hydroxy)azabenzotriazole
HPLC	high pressure liquid chromatography
HRMS	high resolution mass spectrum
HTH	histidine-threonine-histidine
IC_{50}	inhibitor concentration to reduce enzyme activity to 50%
IR	infrared
J	coupling constant
K_a	association equilibrium constant
kDa	kilodalton
K_m	Michaelis-Menten Constant
Leu	leucine
LiHMDS	lithium hexamethyldisilazane

Lys	lysine
m	multiplet, medium
MALDI	matrix assisted laser desorption ionization
MHz	megahertz
MSH	melanocyte stimulating hormone
NMR	nuclear magnetic resonance
NPY	asparagine-proline-tyrosine
[O]	oxidation
Phe	phenylalanine
PAL	peptidylglycine α-amidating lyase
PAM	peptidylglycine α-amidating monooxygenase
PBA	phenylbutenoic acid
PHM	peptidylglycine α-hydroxylating monooxygenase
PHM	proline-histidine-methionine
ppm	parts per million
PYY	proline-tyrosine-tyrosine
q	quartet
rt	room temperature
RTC	rat thyroid carcinoma
s	singlet, strong
Ser	serine
SET	single electron transfer
succ	succinimide
t	triplet
TFA	trifluoroacetic acid
THF	tetrahydrofuran
Thr	threonine

TMS	trimethylsilyl
TOF	time of flight
TRH	thyroid releasing hormone
Tyr	tyrosine
UV	ultraviolet
Val	valine
VIP	vasoactive intestinal peptide
V_{max}	maximum velocity
w	weak

INTRODUCTION

Peptides with *C*-terminal amide functionality ("peptide amides") are an important class of biologically active substances. Many hormones and at least half of all known neuropeptides are peptide amides.[1] They are widely distributed throughout the animal kingdom and the primary *C*-terminal amide functionality is usually essential for biological activity. It is required for high binding affinity to receptors and it may also stabilize the peptide against hydrolysis by proteases.[1] Most peptide amides have neutral amino acids preceding the amide. A partial list of such compounds is found in Table 1.[1]

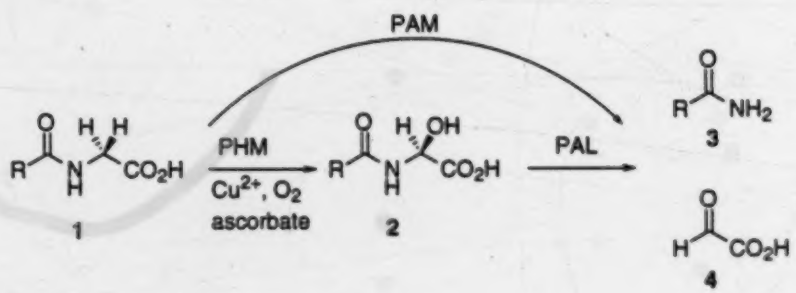
Table 1: Some Peptide Amides[1]

	α-amidated residue	
b,o-CRH; p-galanin; μ-conotoxin	A	alanine
crustacean cardioactive peptide; conotoxins GI, MI, SI	C	cysteine
deltorphin	D	aspartic acid
joining peptide	E	glutamic acid
FMRF-NH_2; gastrin cholecystokinin; γ_1-MSH	F	phenylalanine
oxytocin; vasopressin; GnRH; pancreastatin; leucokinin I leucokinin II	G	glycine
apamin; scorpion toxin II	H	histidine
h,r-CRH; PHI; *Manduca* diuretic hormone; rat neuropeptide EI	I	isoleucine
ELH; cecropin A; conotoxin GIA	K	lysine
β-amidorphin; mastoparan; cecropin B; buccalin;	L	leucine
Substance P; Substance K; PHM; gastrin releasing peptide;	M	methionine
VIP (mammalian); neuromedin U; corazonin;	N	asparagine
calcitonin; TRH	P	proline
melittin; levitide	Q	glutamine
preproglucagon	R	arginine
frog granuliberin-R	S	serine
rat galanin; avian VIP; locust adipokinetic hormone	T	threonine
α–MSH; r,p,h secretin; meorphamide/adrenorphin	V	valine
cockroach myoactive peptide; sea anemone peptide; crustacean erythrophore concentrating peptide	W	tryptophan
NPY; PYY; ω-conotoxin; amylin	Y	tyrosine

Like most biologically active peptides, these substances are biosynthesized from larger precursor proteins. The last step in the biosynthesis involves the conversion of a glycine extended precursor (**1**) to the corresponding peptide amide (**3**) and glyoxylate (**4**).[2,3] The enzyme that accomplishes the synthesis of the amide was first studied by Bradbury *et al.* in 1982.[4] It is now clear that this is a two step process that can be achieved by a bifunctional enzyme, peptidylglycine α-amidating monooxygenase (PAM), or by two separate enzymes, peptidylglycine α-hydroxylating monooxygenase (PHM) and peptidylglycine α-amidating lyase (PAL).[5]

The first step involves hydroxylation of the glycine α-carbon, in a process dependent on oxygen, copper and ascorbic acid.[1] In this step the *pro*-(S) hydrogen is removed and replaced by a hydroxyl with retention of configuration (Scheme 1).[6,7] The α-hydroxyglycine intermediate **2** is relatively stable under physiological conditions and stereospecifically cleaves to the amide product **3** and glyoxylate **4**, in the second, enzyme-mediated step.[6] Labelling studies have shown that the nitrogen of the amide originates from the glycine residue, the oxygen of the hydroxyglycine from molecular oxygen and the glyoxylate aldehyde carbon from the α-carbon of the glycine.[3,6,8] Labelling studies also confirmed that it is the *pro*-(S) hydrogen that is removed.[6]

Scheme 1



More recently, amidating enzymes were shown to catalyse the conversion of *N*-fatty acyl glycines to fatty acid amides and are thereby implicated in the biosynthesis of the sleep inducing hormone, oleamide, in mammals.[9]

Amidating enzymes have been isolated from a number of sources, including honey bee heads and bovine pituitaries (Table 2).[10,11] The shared properties of the enzymes, such as cofactors and substrate requirements, indicate a common mechanism, however the enzymes also exhibit some differences. Especially noticeable is the molecular weight, which varies from 34 to 75 kDa, and the variation in K_m and V_{max}. PHM and PAM have been classified as copper monooxygenases (EC 1.14.17.3) while PAL has no known cofactors and has been classified as class (EC 4.3.2.5). PAM is found in a variety of tissues including the pituitary, hypothalamus, submaxillary and parotid glands, heart atrium, thyroid, gastrointestinal tract and cerebral cortex. Some tumours also have a high level of PAM. PAM activity is primarily located in the secretory granules of the cells of these tissues.[1,2]

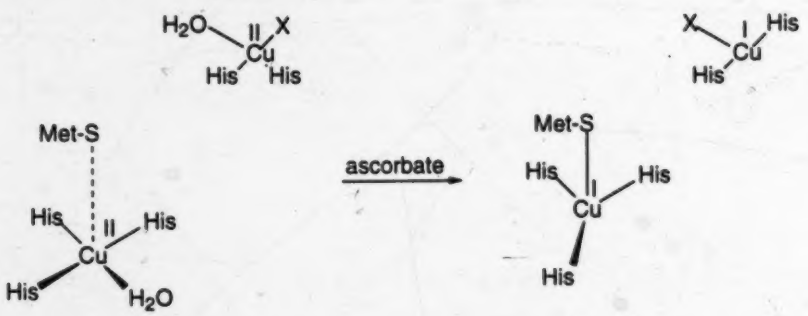
Table 2: A Comparison of Various Isolated PAM Enzymes[10,11]

Tissue Source	Bovine Pituitary	Porcine Pituitary	Frog skin	Rat MTC*	Honey Bee**	Drosophila Fly**
Molecular Weight (kDa)	37 ± 4	64	39	75	63.5	45
Purification (fold)	21000	1000	8000	700	220	-
pH optimum	8.5	6.5-7.5	6.0-7.0	5.0-5.5	6.8	5.0
Vmax (pmol•min/mg)	$1.4x10^6$	$1.35x10^5$	$3.17x10^4$	$4.0x10^6$	$3.8x10^5$	-
K_m (μM)	7	300	0.35	5.6	1.7	2.2
Ascorbate (mM)	1.25	5	0.25	3	1	0.5
Substrate	dYVG	dYVG	Ac-YFG	DnsYVG	DnsFFG	Ac-YVG

*Rat MTC: rat medullary thyroid carcinoma; **contains PHM activity only

PAM is very similar to dopamine beta hydroxylase (DBH). Both enzymes are monooxygenases that require copper, oxygen and ascorbate as cofactors. PAM and DBH have a common catalytic core and 26% conservation of amino acids. Studies on both enzymes show that there are two copper atoms present in the active site.[12] Copper K-extended X-ray absorption fine structure examination of DBH and PAM indicates that there are common histidine rich copper binding sites present (HHM and HTH) and that a methionine is a ligand to one of the coppers when the enzyme is in its reduced form (Figure 1).[13]

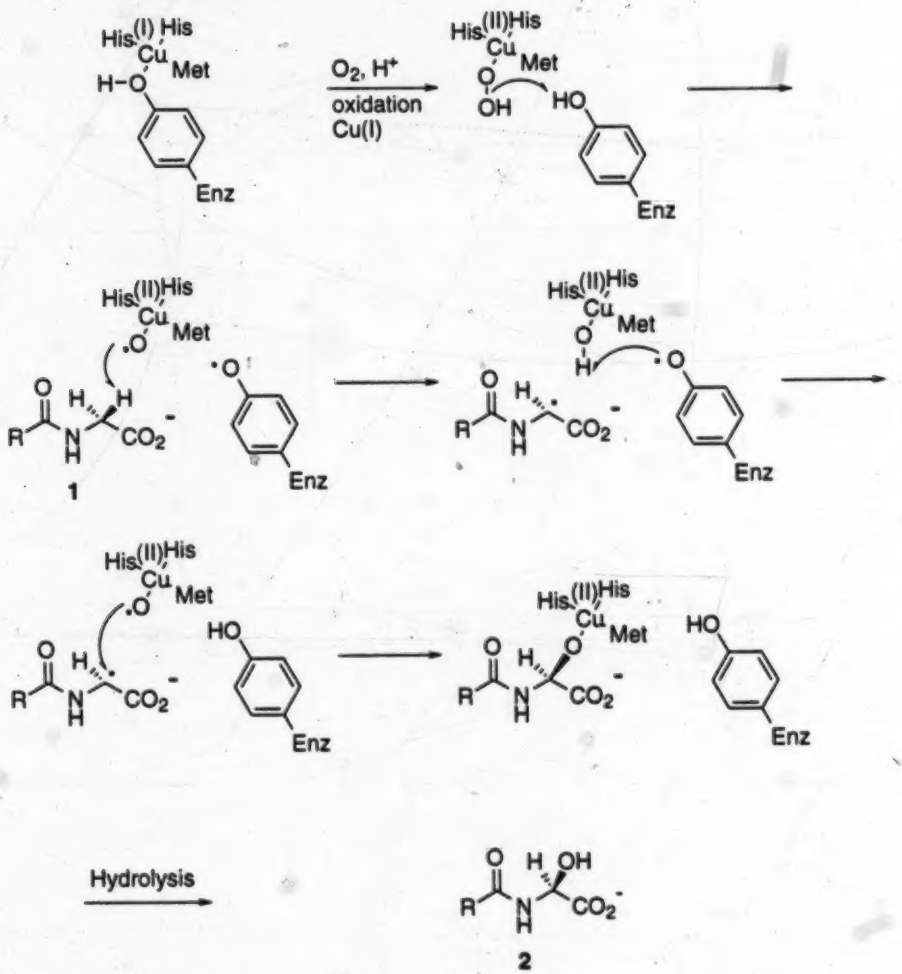
Figure 1: Copper Binding Sites



Mechanistic studies of PAM and DBH[14] suggest that the reaction proceeds *via* a radical mechanism (Scheme 2). Copper (II) is first reduced to Copper (I) by ascorbate; it is then reoxidized to Copper (II) by oxygen to give a copper peroxy species. It appears that a one electron transfer occurs to one of the two coppers, reducing it from copper II to copper I, which then shuttles the electrons to the other copper in the active site. A second electron transfer then occurs to the first copper. It is the second copper which binds the oxygen and is the site of the oxidative hydroxylation. Ascorbate is oxidized to semidehydroascorbate, not dehydroascorbate, which also supports two one electron transfers.[15] Studies with DBH suggest that the peroxy species cleaves to give water and a

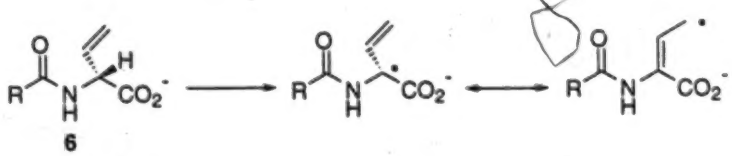
copper-oxy radical prior to reacting with the substrate. These studies also implicate a crucial tyrosine residue.[14] The copper-oxy radical stereospecifically removes hydrogen from the glycine and binds in that position. Hydrolysis then gives the free hydroxyglycine.

Scheme 2: Proposed Mechanism

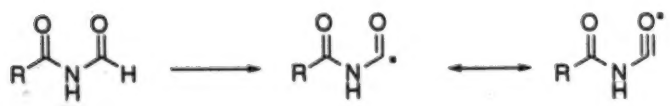


A variety of inhibitors of PAM are now known.[16-21] Benzylhydrazine **5** binds in the ascorbate binding site and irreversibly inactivates the enzyme.[16] Two peptide based inhibitors that bind in the substrate site are the vinylglycine **6** and formylamide **7** derivatives. Both peptides can stabilise a radical that forms during the oxidation and are time dependent, mechanism based irreversible inhibitors (Figure 2).[17,18] Delocalization of the radical that forms at the α-carbon may enhance an electron transfer to Cu (II) or could lead to reaction (i.e. hydrogen abstraction) at another enzyme site. Non-peptide, aromatic formyl amides were also found to be irreversible inhibitors of PAM.[18] A third peptide inhibitor is D-homocysteine compound **8** (IC_{50} 0.04 μM) (Figure 2) that may bind to one of the coppers through its sulfur and also fit in the glycine binding site.[19]

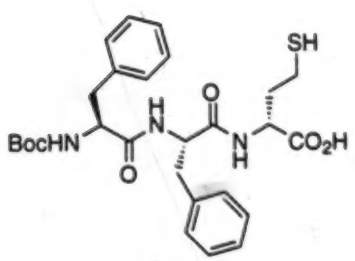
Figure 2: Three Peptide Based Inhibitors



6



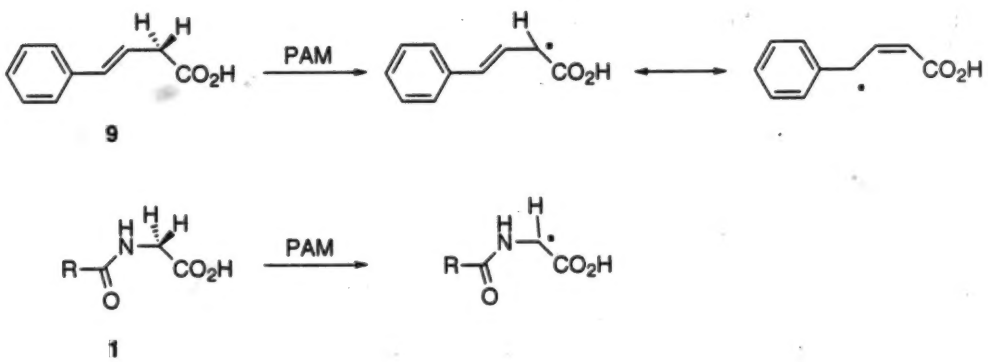
7



8

One of the most important non-peptide inhibitors is *trans*-4-phenyl-3-butenoic acid **9** (PBA);[20] with an IC_{50} of 0.4 μM it is one of the best inhibitors of PAM. It is a time dependent, mechanism based irreversible inhibitor, which requires all cofactors for inhibition. The enzyme can be protected by its natural substrate. The PBA **9** appears to covalently bind to the enzyme, because upon inhibition with ^{14}C labelled PBA the radioactivity coelutes with the enzyme on a Sephadex column.[20] Presumably the aromatic group takes advantage of the hydrophobic pocket in the active site and the *trans* double bond mimics the *trans* amide functionality of glycine (Figure 3). A closely related inhibitor is *trans*-styrylthioacetic acid **10** (Figure 4).[21]

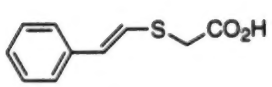
Figure 3: PBA as an Inhibitor of PAM



9

1

Figure 4



10

A structure activity study with some unsaturated organic acids has been reported (see Table 3 for selected results)[22] and some trends were deduced from the results. The

carbon alpha to the acid functionality should be unsubstituted.[22] The potential inhibitor should not contain any highly charged or polar groups other than the necessary carboxylate.[22] If the acid can bind in the active site, a double bond in the α-β position or the β-γ position, relative to the carboxylate group, is enough to allow the compound to inactivate PAM, however the β-γ analogues are much more potent inhibitors. The amide functionality of the natural substrate can be replaced by groups other than double bonds, for example aromatic groups greatly aid in binding of the inhibitor to the active site.[22]

Table 3: Structure Activity Relationships Among Inhibitors of PAM

Unsaturated Acid	50% Competition (mmol)	50% Inactivation (mmol)	C_{50}/I_{50}
trans-2-Pentenoic acid	74.98	20.96	3.58
trans-2-Hexenoic acid	46.46	28.13	1.65
trans-2,4-Hexadienoic acid	11.09	34.99	0.32
trans-2,4,6-Octatrienoic acid	5.67	3.86	1.47
Vinylacetic acid	13.06	6.36	2.05
4-Phenyl-3-butenoic acid	0.031	0.011	2.82
Phenylpropiolic acid	48.62	39.92	1.22

The mode of inhibition of PBA is unknown. The goal of this project is to gain insight into the inhibition of PAM by phenylbutenoic acids and thereby increase our understanding of the mechanism of amidation. This problem was investigated on three fronts. Firstly, PBA was incorporated into a peptide to attempt to determine how it binds in the active site. Secondly, labelled analogues of PBA were synthesized to investigate the possibility of covalent attachment of the inhibitor to the enzyme. Finally a series of

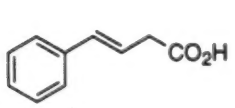
substituted phenylbutenoic acids were synthesized for a structure activity study to gain more insight into the active site requirements of the enzyme.

Inhibitors of PAM are not only mechanistic probes, but also have potential as insect control agents, drugs to control hormone or neuropeptide formation or compounds to influence fatty acid amide induced processes such as sleep.

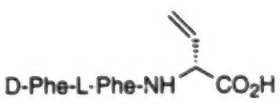
RESULTS AND DISCUSSION

Synthesis of Tripeptide Inhibitors Containing D- and L-Styrylglycine

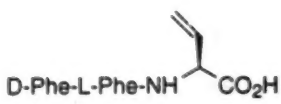
Previous work has demonstrated that *trans*-4-phenyl-3-butenoic acid **9** and the tripeptide D-Phe-L-Phe-D-vinylglycine **6** are mechanism based irreversible inhibitors of PAM.[5,11,20] In contrast, the diastereomeric tripeptide D-Phe-L-Phe-L-vinylglycine **11** is only a weak competitive inhibitor. Since the *pro*-S hydrogen is removed during the hydroxylation of the substrate, it is expected that the D-vinylglycine analogue **6** would be a better inhibitor because it has a hydrogen in the correct location available to the enzyme, in contrast to the L-isomer **11**. Loss of hydrogen presumably leaves a delocalized radical at the methylene carbon (Figure 4).



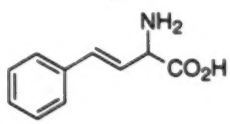
9



6



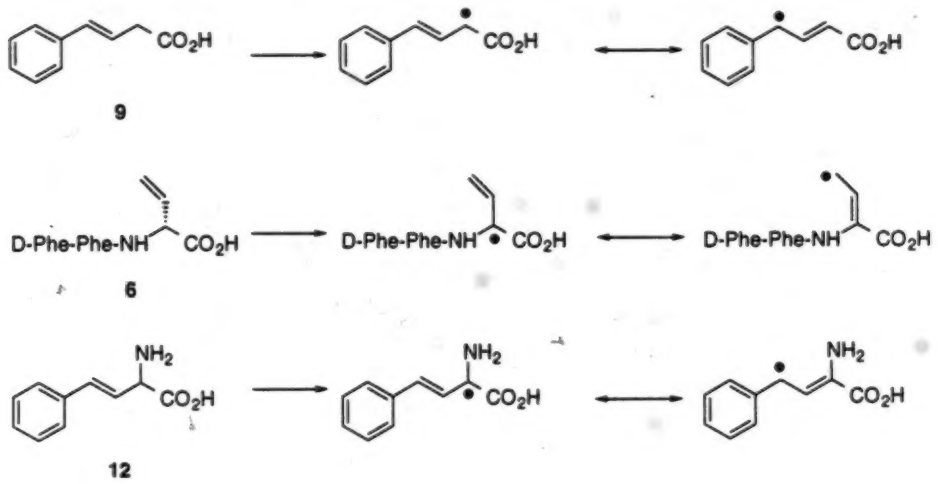
11



12

A hybrid of PBA **9** and D-Phe-L-Phe-D-vinylglycine **6** was chosen as a target. It contains the unnatural amino acid (*E*)-2-amino-4-phenyl-3-butenoic acid (styrylglycine) **12**. The primary target was the tripeptide D-Phe-L-Phe-D-styrylglycine, since it has both the substrate peptide backbone and a substituted vinylglycine with the hydrogen in the correct position for the enzyme abstraction. The double bond and adjacent phenyl ring allow delocalization of radical character from the α carbon (Figure 4).

Figure 4: Delocalization of Radical Character in the Mechanism-Based Inhibitors



This target compound is also a probe of the mode of substrate binding in the active site. It has been assumed that the double bond in PBA **9** mimics the primary amide functionality of a peptidyl glycine.[22] This, however, is unlikely to be the case for the peptidylvinylglycine inhibitor **6**. Presumably the amide functionality in this inhibitor is recognized by the enzyme and the vinyl group must fit in another part of the active site. It is possible that PBA **9** binds in the same position in the active site, i.e. as a phenyl substituted vinylglycine analogue. If the target peptide is as good a mechanism based inhibitor as PBA **9**, it would suggest that PBA **9** binds in the same way as the vinylglycine **6**.

Despite the fact that styrylglycine was first described in 1889, very few syntheses of the free amino acid have been published.[23-25] This is presumably due to the tendency of the double bond to shift into conjugation with the carboxyl group under basic conditions.[26] Only two reports describe the synthesis of non-racemic material. Sakota and co-workers reported the synthesis of L-styrylglycine by the enzymatic hydrolysis of

racemic *N*-acetylstyrylglycine,[24] whereas Baldwin *et al.* used a sequence involving condensation of benzaldehyde with a dianion derived from L-aspartic acid, followed by decarboxylative dehydration, to generate L-styrylglycine as a mixture of (*E*) and (*Z*) isomers.[25] The latter method was chosen for this synthesis as it seemed that the (*E*) and (*Z*) isomers would be separable. This route would therefore allow access to both enantiomers of styrylglycine in a controlled manner from D- and L-aspartic acids.

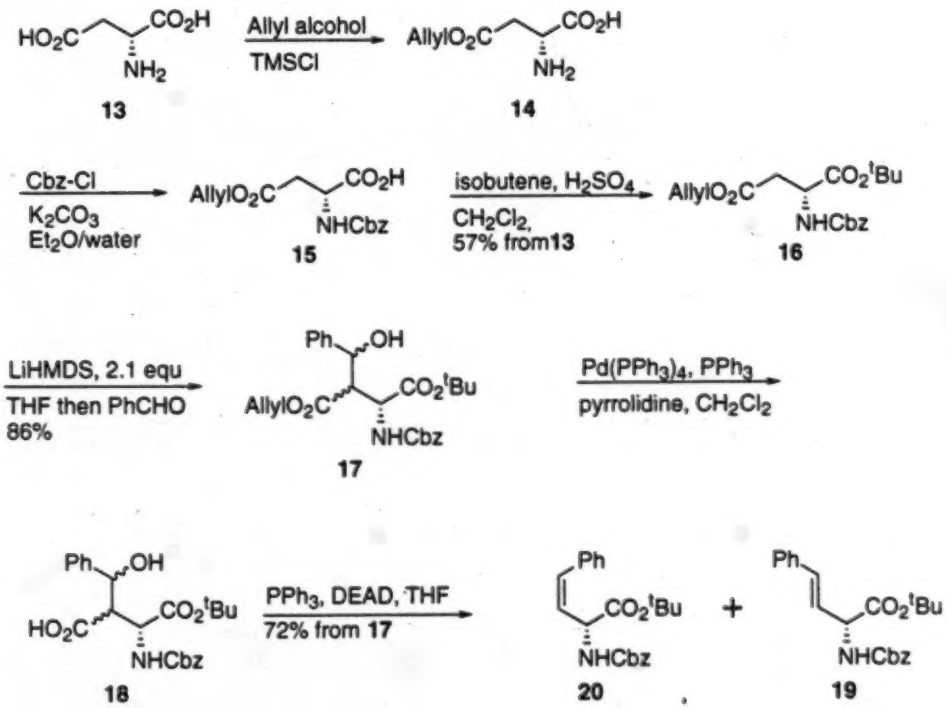
Synthetic Studies

The carboxylic acid of the side chain of D-aspartic acid **13** is esterified to give the allyl ester **14**.[25] The amino group is protected using the carboxybenzyl group **15**.[25] The remaining carboxylic acid is converted to the *tert*-butyl ester to give the fully protected D-aspartate **16** with an overall yield of 57% over three steps.[25] Treatment of the ester **16** with 2.1 equivalents of LiHMDS, followed by condensation with benzaldehyde, gives the aldol product **17** in 86% yield, as a 2.1:1 mixture of two diastereomers. It was possible to isolate the major isomer at this stage by simple column chromatography, but this proved to be unnecessary. Deprotection of the allyl ester using a palladium catalyst in the presence of pyrrolidine proceeds to give the free side chain carboxylic acid **18**.[25] Treatment of this hydroxy acid **18** product with triphenylphosphine and diethyl azodicarboxylate (DEAD) gives a 2.9:1 mixture of the (*E*) **19** and (*Z*) **20** alkenes in a combined yield of 72% (Scheme 3). The same 2.9:1 ratio of alkene isomers is generated starting from either the pure major diastereomer of diester **17**, or from a mixture of diastereomers. This shows that the decarboxylative dehydration does not occur in a stereospecific manner.

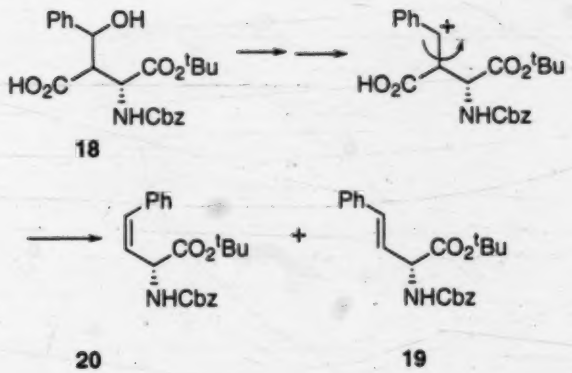
It has been previously reported that the decarboxylative dehydration of β–hydroxy acids with triphenylphosphine and DEAD occurs in a stereospecific *anti*- fashion.[27] The non-stereospecific reacton in this case may be due to the phenyl substituent on the hydroxyl carbon. Activation of the hydroxyl group followed by the loss of

triphenylphosphine oxide would generate a relatively stable benzylic carbocation which, upon rotation and loss of carbon dioxide, would generate a mixture of (*E*) and (*Z*) alkenes, with the (*E*) alkene predominating for steric reasons (Scheme 4).

Scheme 3

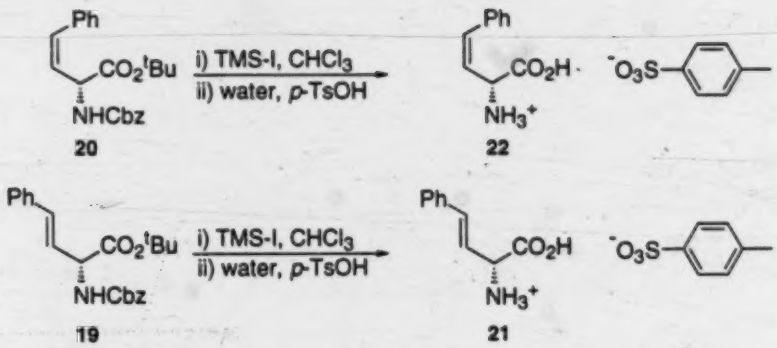


Scheme 4



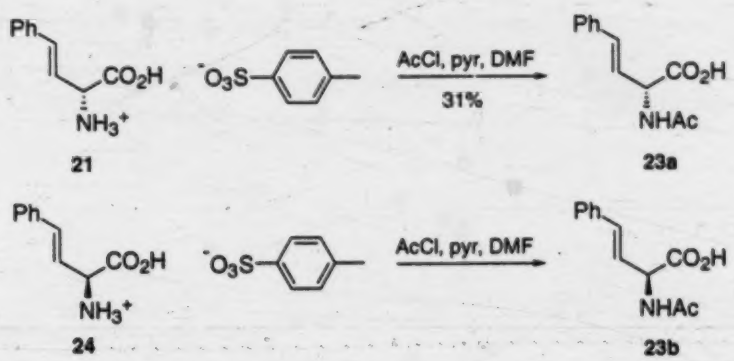
Separation of the alkene isomers is possible by careful column chromatography using toluene as an eluent. Subsequent deprotection of alkene **19** to the free amino acid is readily effected using excess trimethylsilyl iodide in chloroform, followed by quenching with water and *p*-toluenesulfonic acid. After recrystallisation from methanol/ether, and purification of the residue by reverse phase HPLC, D-styrylglycine **21** is obtained in a yield of 71%. Similar deprotection of the (*Z*)-alkene **20** gives a 93% yield of the corresponding (*Z*)-amino acid **22** (95% purity), which was not purified further. Repetition of the entire sequence, starting with the L-aspartic acid, affords (*E*)-L-styrylglycine as its *p*-toluenesulfonate salt (Scheme 5).

Scheme 5



Treatment of D-styrylglycine **21** with acetyl chloride and pyridine in DMF converts it to the *N*-acetyl derivative **23a**. Similar reaction of L-styrylglycine affords *N*-acetyl-L-styrylglycine **23b** (Scheme 6).[28]

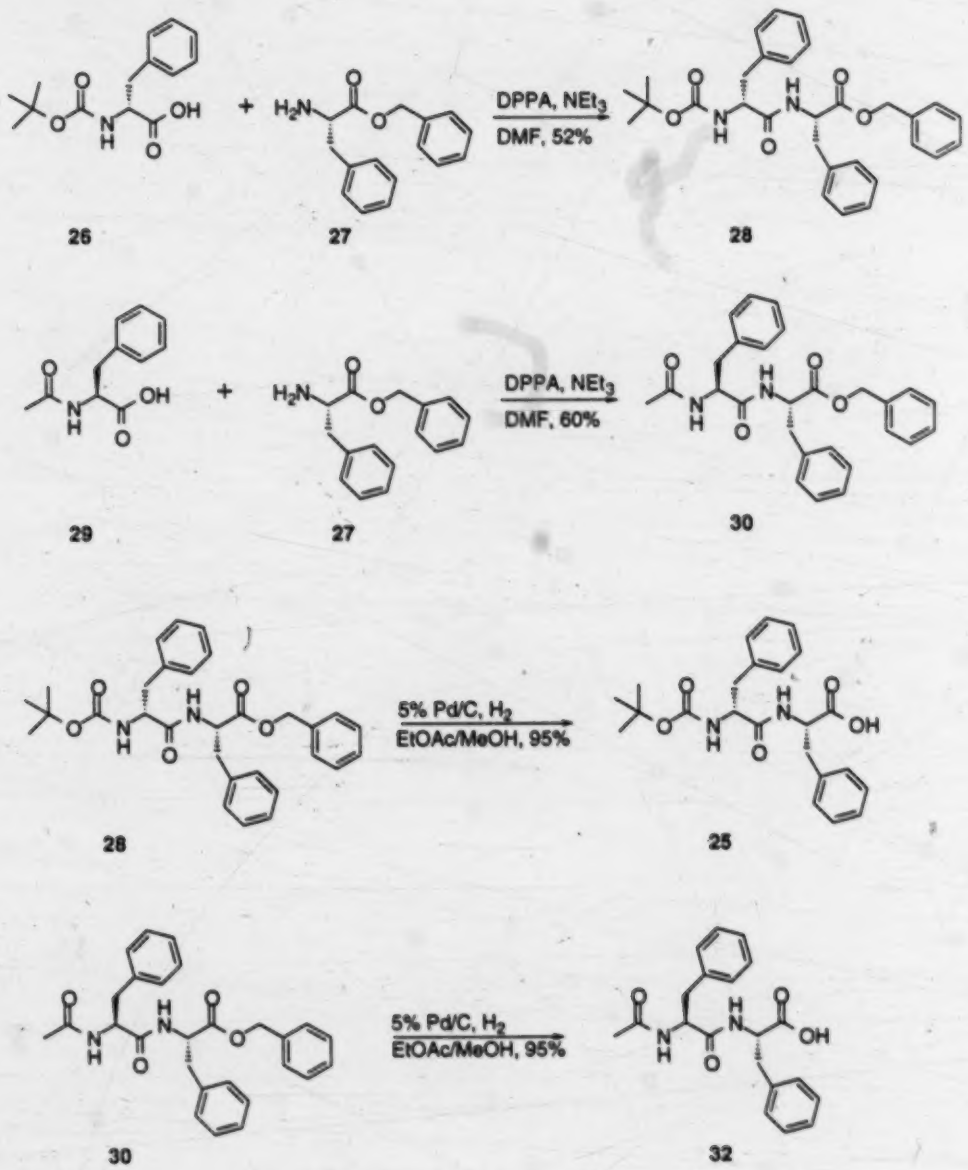
Scheme 6



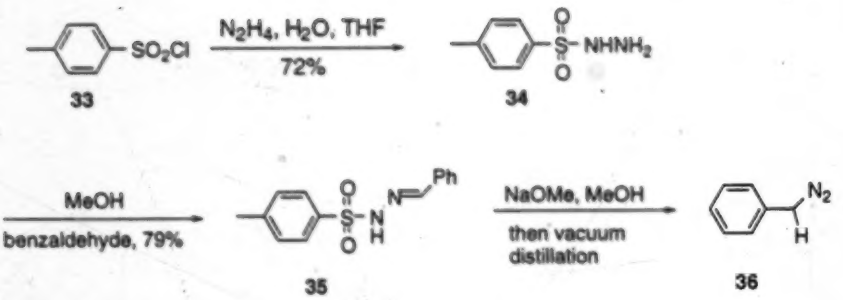
Two separate dipeptides were prepared using standard techniques to couple to the styrylglycines. Previous work with the vinylglycine series had used Boc-D-Phe-L-Phe **25**,[6] a second peptide *N*-Ac-L-Phe-L-Phe **32** was also prepared (Scheme 7). In a first attempt to couple D-styrylglycine **21** to the dipeptide, the benzyl ester was prepared *in situ* with phenyldiazomethane (Scheme 8) and then immediately coupled to the dipeptide.

This route was unsuccessful and another protecting group was sought for the styrylglycines.

Scheme 7

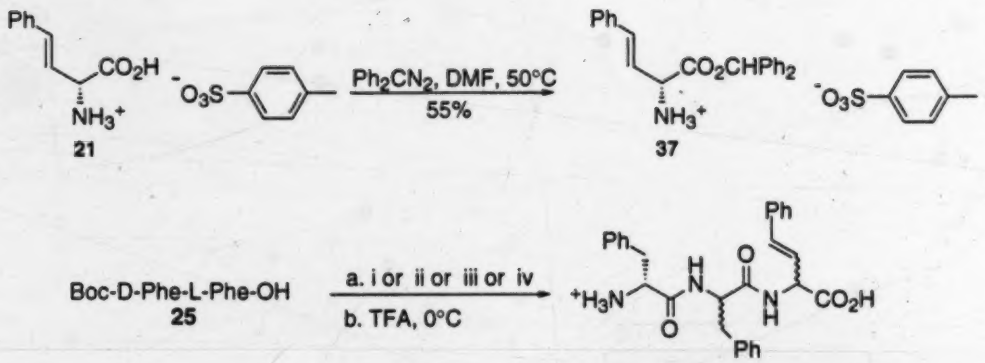


Scheme 8



A successful route to the desired peptide was completed by Dr. Mark Andrews. The target, D-styrylglycine **21** was first protected as the acid labile diphenylmethylester **37**, by reaction with diphenyldiazomethane in DMF.[29] Activation of the dipeptide Boc-D-Phe-Phe-OH **25** with BOP reagent in the presence of triethylamine followed by reaction with the amino ester **37**, gives a protected tripeptide.[30] Chromatographic purification and subsequent deprotection using TFA generates the desired tripeptide (Scheme 9, conditions i). Further purification by reverse phase HPLC and analysis by NMR spectrometry indicated that this coupling/deprotection procedure generates four diastereomeric tripeptides **38-41** in a combined yield of 64% (Table 4).

Scheme 9



i. BOP reagent, Et_3N, DMF, 0°C then **37**, DMF, Et_3N
ii. HOAT, EDC•HCl, DMF, CH_2Cl_2, then **37**, DMF, collidine
iii. HOAT, EDC•HCl, DMF, then **21**, collidine
iv. HOAT, EDC•HCl, DMF, then L-styrylglycine *p*-toluenesulfonate salt, collidine

Table 4: Yields of Diasteromeric Tripeptides **38-41** Under Different Coupling Conditions

Conditions	Yield **38** (%)	Yield **39** (%)	Yield **40** (%)	Yield **41** (%)
i	12	15	12	25
ii	43	33	≤1	≤1
iii	19	≤1.5	≤1	≤1
iv	≤2	20	≤1	≤1

These four products presumably arise by epimerisation of the carboxyl terminal residue of the activated dipeptide, during or following coupling of the diastereomeric dipeptides to the styrylglycine ester. Protected styrylglycine derivatives are known to

racemise rapidly under basic conditions,[15] so it is possible that ester **37** racemises under the reaction conditions, or ultimately, that the protected tripeptides epimerise after coupling.

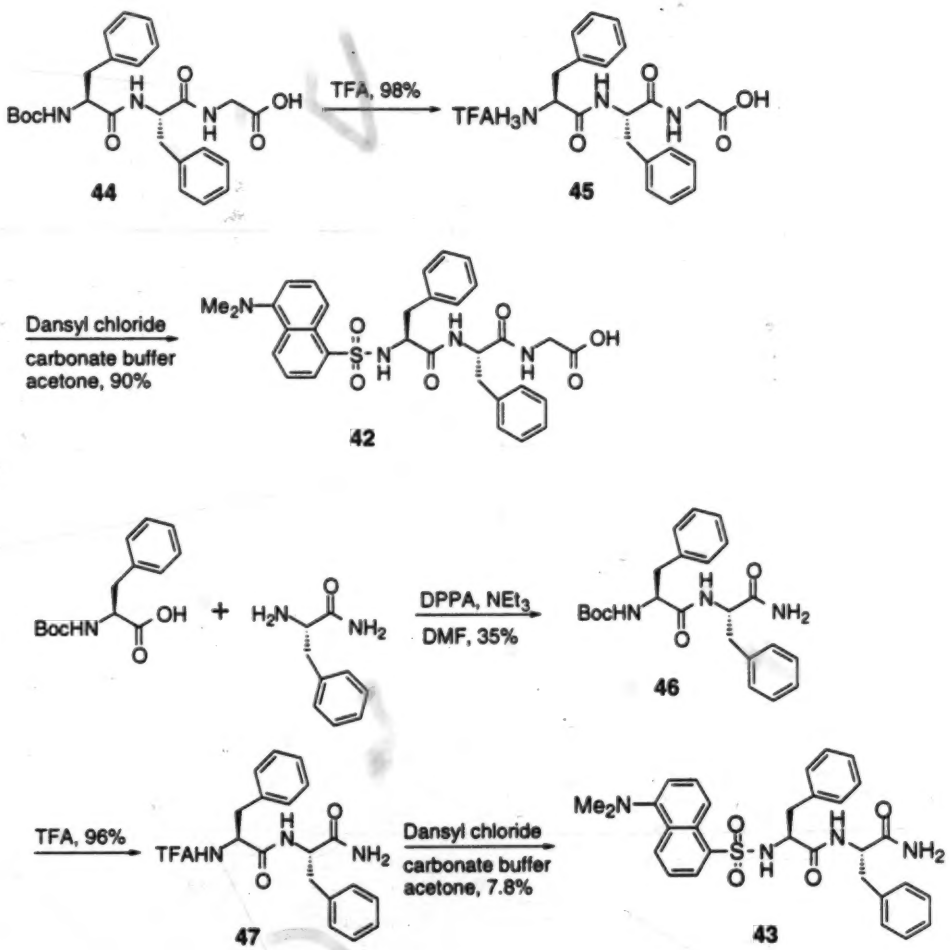
In an attempt to form only the single tripeptide **38**, Boc-D-Phe-L-Phe-OH was activated as its 1-(hydroxy)azabenzotriazole (HOAT) ester and treated with **37** in the presence of collidine (Scheme 8, conditions ii), as this combination is known to minimise epimerisation in sensitive peptide coupling reactions.[31] This strategy suppresses epimerisation of the activated dipeptide but does not prevent epimerisation of the styrylglycine residue. Tripeptides **38** and **39** are isolated as a 4:3 ratio in 76% yield after acidic deprotection. In the absence of collidine the same ratio of the two peptides is formed, although in lower yield.

Fortunately, formation of a single tripeptide can be achieved by treatment of the HOAT ester of Boc-D-Phe-L-Phe-OH with the amino acid salt **21**, in the presence of collidine (Scheme 4, conditions iii). After deprotection with TFA, this affords the tripeptide **38** in a low, 19% yield, but does allow its definite identification as D-Phe-L-Phe-D-styrylglycine. From analysis of the crude reaction product by HPLC, the diastereomeric excess (d.e.) of peptide **38** is estimated as >86%. This indicates that the enantiomeric excess (e.e.) of the D-styrylglycine **21** is >86%. Reaction of the activated ester of BocD-Phe-L-Phe-OH with L-styrylglycine, in a similar manner (Scheme 9, conditions iv), generates tripeptide **39** as the only isolated product, thereby allowing confirmation of its identity as D-Phe-L-Phe-L-styrylglycine. The same procedure with the activated ester of Boc-D-Phe-D-Phe-OH permits identification of the diastereomeric tripeptides **40** and **41.**

Biological Studies

The four tripeptides **38-41** were tested individually by Dr. Mark Andrews, along with both enantiomers of *N*-acetylstyrylglycine **23a** and **23b**, as inhibitors of α-amidating activity using recombinant PAM from rat medullary thyroid carcinoma.[32] The inhibition studies were done using an HPLC assay similar to that published previously.[5] Assays were performed using 56 ng of PAM and the enzyme was incubated at 37°C with the appropriate concentration of inhibitor in a shaker bath for 1 hour. *N*-Dansyl-L-Phe-L-Phe-Gly **42** (see scheme 10 for preparation of the enzyme substrate **42** and product **43**) was then added to give a substrate concentration of 50 μM and a total volume of 100 μL and the solution was returned to the shaker bath. After 1 hour the enzymatic reaction was terminated by the addition of 10 μL ethylenediamine tetraacetic acid (500 mM, pH 8). The product amide was separated by reverse phase HPLC and quantified by comparing the peak area with a standard curve constructed by serial dilution of an authentic sample of *N*-dansyl-L-Phe-L-Phe-NH_2 **43**. All assays were performed at least in duplicate, and percentage inhibition was determined by comparison with an identical assay done in the absence of inhibitor.

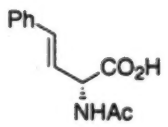
Scheme 10



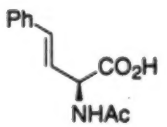
The results show that these vinylglycine-phenylbutenoic acid analogues are not irreversible inactivators nor do they significantly inhibit *C*-terminal amide formation at submillimolar levels (Table 5), although the tripeptide inhibitors are more potent than either enantiomer of *N*-acetylstyrylglycine.

Table 5: Approximate IC_{50} Values for PAM Inhibitors

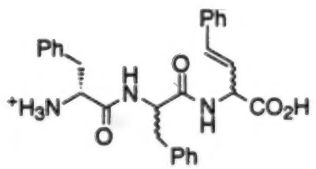
Compound	23a	23b	38	39	40	41
IC_{50} (mM)	1.5	1.0	0.4	0.45	0.1	0.3



23a



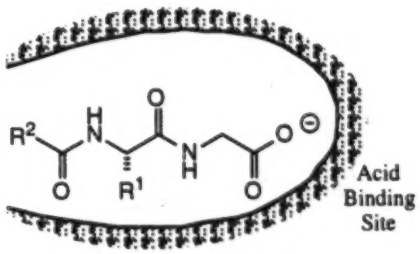
23b



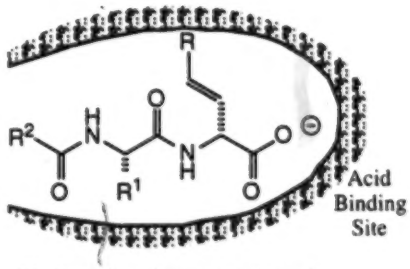
38 D-Phe-L-Phe-D-styrylglycine
39 D-Phe-L-Phe-L-styrylglycine
40 D-Phe-D-Phe-L-styrylglycine
41 D-Phe-D-Phe-D-styrylglycine

Despite the fact that (*E*)-4-phenyl-3-butenoic acid **9** and the tripeptide D-Phe-L-Phe-D-vinylglycine **6** are both micromolar mechanism-based irreversible inactivators of PAM,[5,11,20] none of the four tripeptides **38-41** containing D- or L-styrylglycine display potent inhibition of this enzyme. From this we conclude that the phenyl group on the styryl sidechain of these peptides is too large to fit into the enzyme active site. This implies that 4-phenyl-3-butenoic acid (**9**) adopts a conformation in the enzyme active site such that the double bond in the 4-phenyl-3-butenoic acid **9** acts as a mimic of the planar amide linkage in a peptide (Figure 6). The fact that neither enantiomer of *N*-acetyl styrylglycine, **23a** and **23b**, inhibits PAM indicates that even an acetylamino substituent is too large a sidechain to be accepted α to the carboxyl terminus. This is in agreement with earlier work which found that a cyclopropyl group is also too large to be tolerated.[17]

Figure 6: Possible Orientation of PAM Inhibitors in the Enzyme Active Site

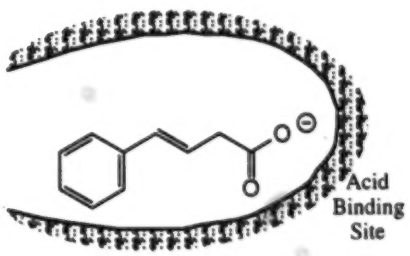


A: Binding of a substrate molecule in the active site of PAM.



B: Binding of a substrate analogue in the active site of PAM.

R = H, accepted; R = Ph, excluded.



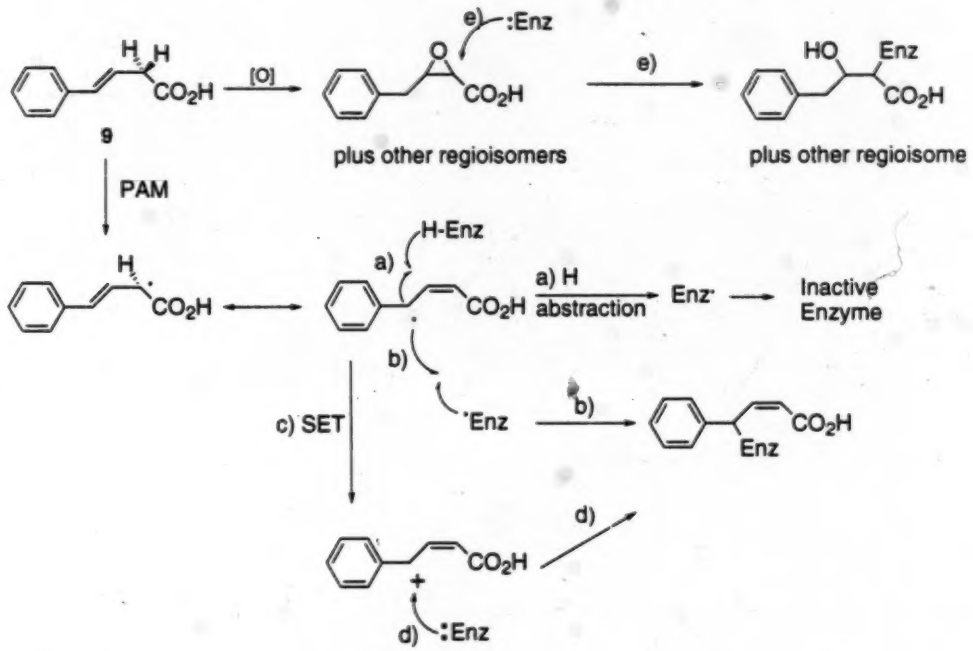
C: Binding of phenyl-butenoic acid **4** in the active site of PAM

Synthesis of Labelled Inhibitors of PAM

Phenylbutenoic acid is one of the best known mechanism based, irreversible inhibitors of PAM.[3] It has been presumed that this inhibitor works by allowing delocalization of radical character from the methylene carbon leading to enzyme inactivation (Scheme 11). The first step of the inhibition is most likely the abstraction of a hydrogen from the methylene carbon. Several things could happen from this point. The delocalized radical can covalently combine with the enzyme (path b) or the radical could abstract a hydrogen from the enzyme and deactivate it (path a) without covalently attaching to it. A single electron transfer could occur to give the cation (path c) and this cation could be attacked by a nucleophile in the active site (path d). Alternatively, the intermediate copper-oxo species may oxidize the double bond to give the epoxide that would be susceptible to attack by a nucleophile in the active site (path e).

The mode of inhibition of PAM by phenylbutenoic acid was investigated using labelled analogues of PBA. The immediate goals were: i) to determine if the inhibitors are covalently bound in the enzyme active site; and ii) determine the mode of attachment of the inhibitor. The crystal structures of PAM and DBH are unknown, although some information about residues in the active site of DBH has been obtained through the use of site specific mutagenesis.[13,14] However very little is known about the active site of PAM.

Scheme 11: Possible modes of inhibition of PAM by phenylbutenoic acids

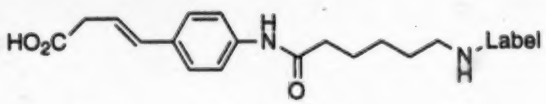


The basic strategy for achieving these objectives centers on attachment of a "label" to the phenylbutenoic acid. This moiety should permit spectrometric (i.e. UV, MS, fluorescence) detection of attachment of the inhibitor to protein residues and/or should assist separation of peptide-inhibitor complexes by affinity chromatography (Figure 7). The labels were attached to phenylbutenoic acid *via* a 6 carbon spacer, to minimize interference with the inhibitor binding to the enzyme. Ideally, the label should be accessible on the surface of the enzyme for a recognition event.

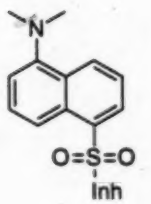
In the present design, the labels are placed in the *para* position of the aromatic ring in the hope that they will not interfere with the inhibition of PAM by PBA. The fluorescent labels, such as fluorescein, dansyl and 2,6-DMANS, have unique fluorescence and UV absorption properties not displayed by the enzyme. Dabsyl is a highly coloured moiety that has a characteristic visible spectrum which permits facile identification.

Biotin, a vitamin, binds with a high affinity to avidin, a carrier protein. The avidin-biotin interaction is the strongest known noncovalent association ($K_a = 10^{15}\ M^{-1}$) between protein and ligand.[50] This interaction could be used to determine if the inhibitor is covalently attached to PAM and could also be employed in affinity-based separation.

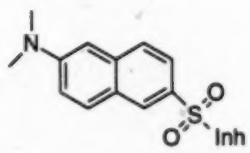
Figure 7: Design of Labelled Inhibitors



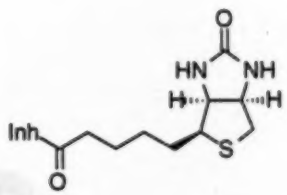
Labels



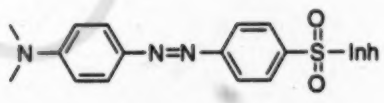
Dansyl



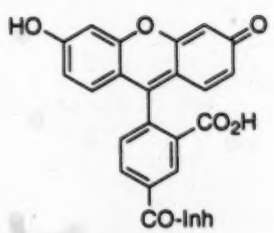
2,6-DMANS



Biotin



Dabsyl



Fluorescein

Following inhibition of PAM by phenylbutenoic acid analogues, the inhibited enzyme could then be proteolytically digested into a large number of peptide fragments. These fragments could be purified, and the fragment containing the inhibitor identified and separated using the unique properties of the label. The peptide fragment can in principle be identified from its mass, based on residue specificity of the protease used to digest PAM and subtracting the mass of the inhibitor from that of the fragments. This

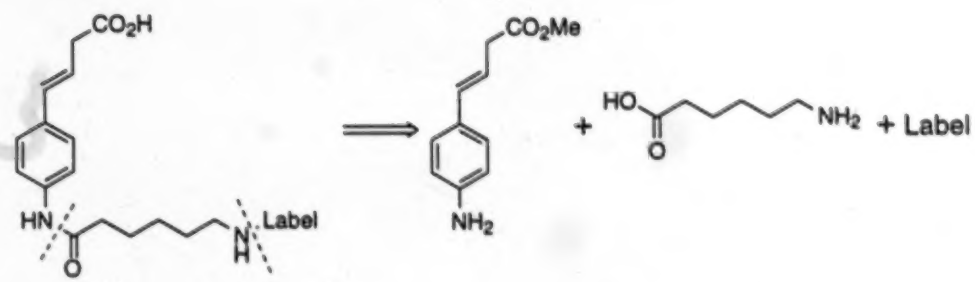
identification could presumably be verified by a number of techniques, including sequencing the peptide.

These labelled analogues of PAM could also possibly be used as a probe to determine in what types of cell tissues PAM is concentrated and identify in what cellular substructures the enzyme is localized.[11b] This could be done by placing the labelled inhibitor in the surrounding media of cells or tissues. If the cells take up the inhibitor and the inhibitor forms a covalent attachment with PAM, the site could potentially be identified and visualized by the use of fluorescence microscopy,[33] or ELISA techniques.[34]

Synthesis of Labelled Inhibitors

The labelled inhibitors can be seen to be composed of three parts (Figure 8). The first part is a *para* amino-4-phenyl-3-butenoic acid moiety, protected as the methyl ester. The second part is the spacer, a six carbon chain that contains an acid group to join to the *para* amino group of the phenylbutenoic acid and an amine to attach the label. The third piece is the label that contains an activated group to attach to the amine of the spacer.

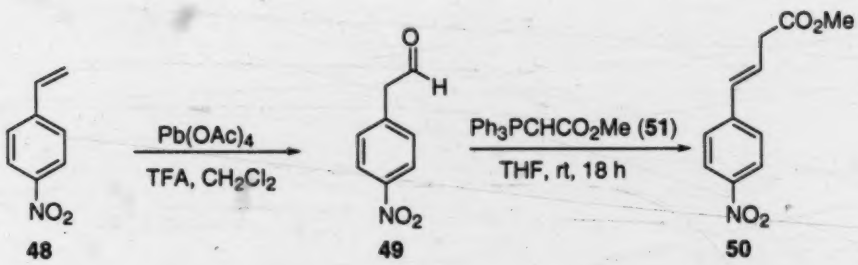
Figure 8



The phenylbutenoic acid portion of the molecule can be synthesized form *para*-nitrostyrene, (Scheme 12). *Para*-nitrostyrene **48** can be oxidized to the

phenylacetaldehyde **49** with lead tetraacetate and trifluoroacetic acid in 79% yield.[35] A Wittig reaction yields the methyl ester of *para*-nitrophenylbutenoate **50** (80%). This synthesis is discussed in more detail in the following section.[36]

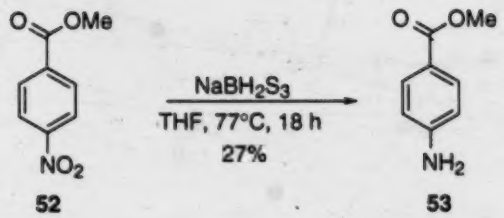
Scheme 12



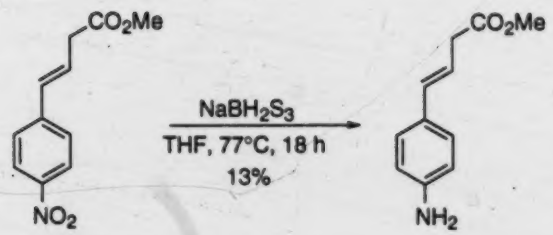
48 **49** **50**

Reduction of the nitro group in the presence of a double bond, an ester and an aromatic ring was expected to be troublesome, however a review of the literature indicated that sulfurated sodium borohydride is capable of this.[37] As a test, methyl *para*-nitrobenzoate (**52**) was reduced to the amine **53** in only a moderate yield of 27%.[37] The yield was even lower (13%) when methyl *para*-nitrophenylbutenoate (**50**), was reduced using the same reaction conditions (Scheme 13). Amine **54** decomposes on silica gel, but Florisil was found to allow chromatographic purification without decomposition.

Scheme 13



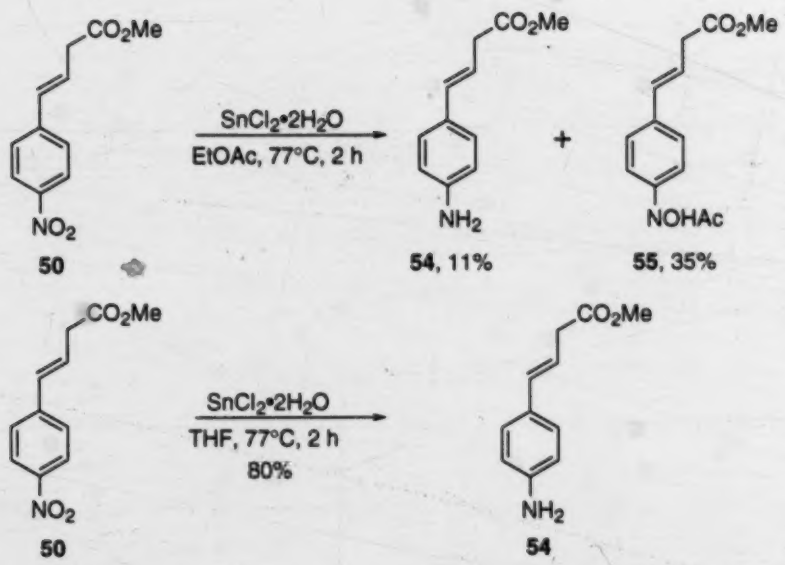
52 **53**



50 **54**

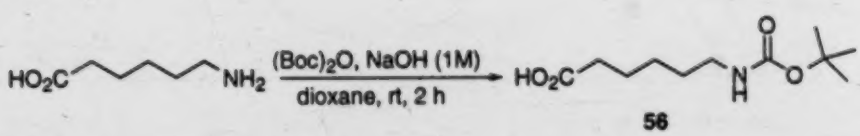
Stannous chloride is also capable of reducing nitro groups in the presence of other reducible functionality.[38] Following the literature procedure, using ethyl acetate as a solvent, only a small amount (11%) of amine **54** was recovered. The major product was found to be compound **55** in which the intermediate *N*-hydroxyamino group had been acetylated, presumably by ethyl acetate. This can be prevented by switching to THF as a solvent, which allows a good yield (80%) of the amine **54** to be obtained (Scheme 14).

Scheme 14



The spacer, 6-aminocaproic acid (**55**), is commercially available. The amino group can be protected with either a Boc or a Cbz group, in order to allow the spacer to be coupled to the *para*-amino-phenylbutenoic acid **54** (Scheme 15). The amino group of **55** reacts with Boc anhydride in the presence of sodium hydroxide to give the protected derivative **56** in 62% yield.[39]

Scheme 15

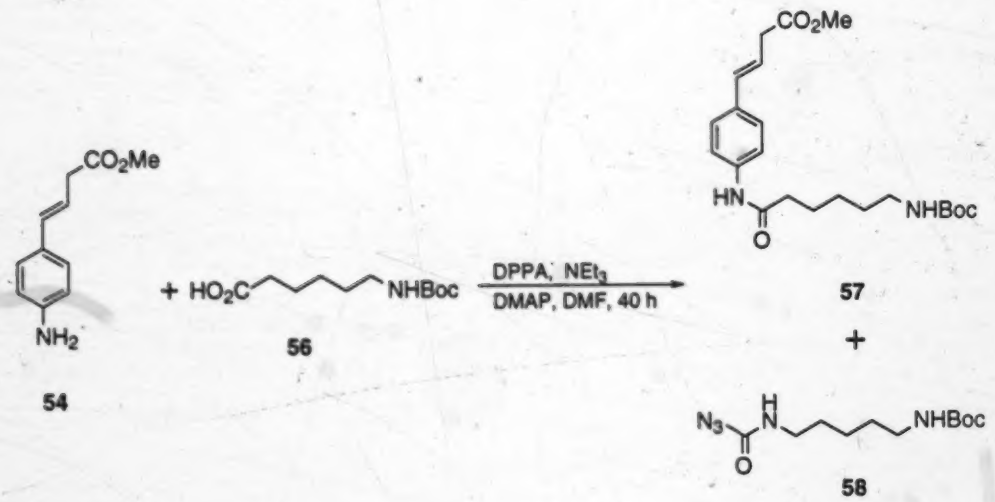


56

The next step in the synthesis is to couple the protected spacer to the amine **54**. The first method examined used diphenylphosphoryl azide (DPPA) as a coupling agent,[40] and was found to be very slow (>48 hours) and ineffecient (22% yield of **57**) (Scheme

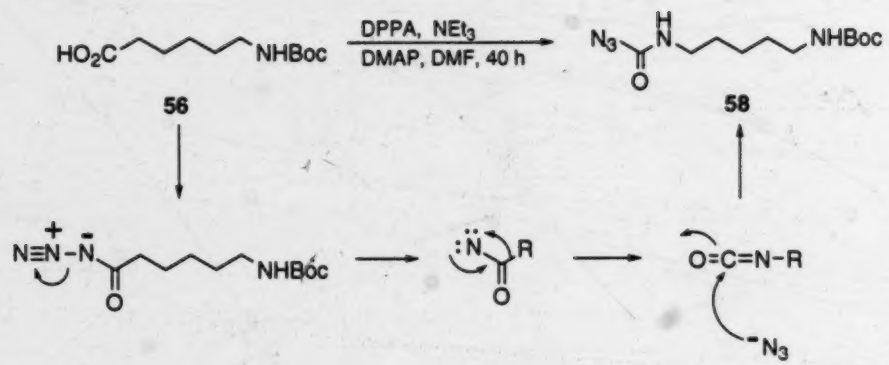
16). Another compound **58** was isolated as the major product (37%) from the coupling reaction (Scheme 16). A Curtius type rearrangement can be invoked to explain the formation of **58** (Figure 9).[43] Amine **54** is not very nucleophilic as its lone pair can be delocalized into the aromatic system. Reaction of the amine with the acyl azide, formed by DPPA activation, is therefore sluggish. The acyl azide is not stable and rearranges in a Curtius fashion to give **58**. Such rearrangement usually requires heat, however the extended reaction times at room temperature are apparently sufficient to allow this process to occur.

Scheme 16



The *N*-Cbz-6-aminocaproic acid was also coupled to the amine **54** with DPPA as a coupling agent, to give 21% of the corresponding product **59.**

Figure 9



This DPPA coupling procedure was abandoned as the activated acyl azide was not stable enough to withstand the long reaction times necessary to complete the peptide coupling reaction without competing rearrangement.

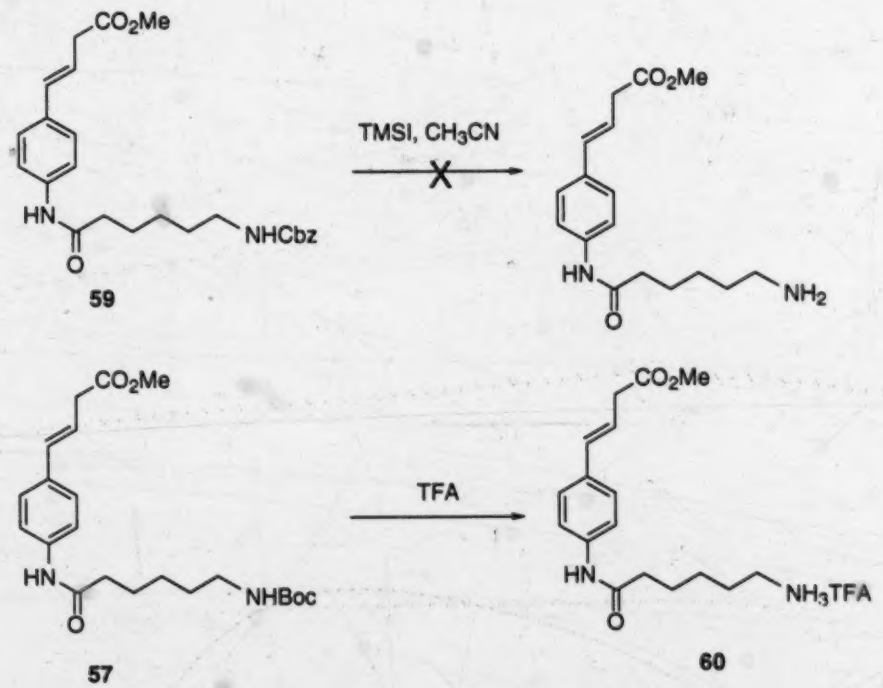
EEDQ (2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline) is another coupling agent known for activating acids with little or no racemization.[42] This suggests that the activated form of the acid should be stable to long reaction times. The Boc protected spacer **56** and amine **54** were coupled using EEDQ to give **57** in 27% yield. Again, the reaction proceeded very slowly, taking 4 days to give this low yield of product.

Both coupling procedures resulted in another undesired side reaction. The use of base during coupling resulted in partial isomerization of the double bond to the α,β unsaturated ester. The β,γ, and α,β-isomers were difficult to separate and were not segregated at this stage as it was hoped that they could be isomerized back to the desired position later.

The next step required removal of the protecting group from the amine. The Boc group was removed with trifluoroacetic acid to give the free amine **60** in 95% yield (Scheme 17).[43] The Cbz group is normally removed by hydrogenation, however this would probably reduce the double bond. Another method for removing Cbz groups

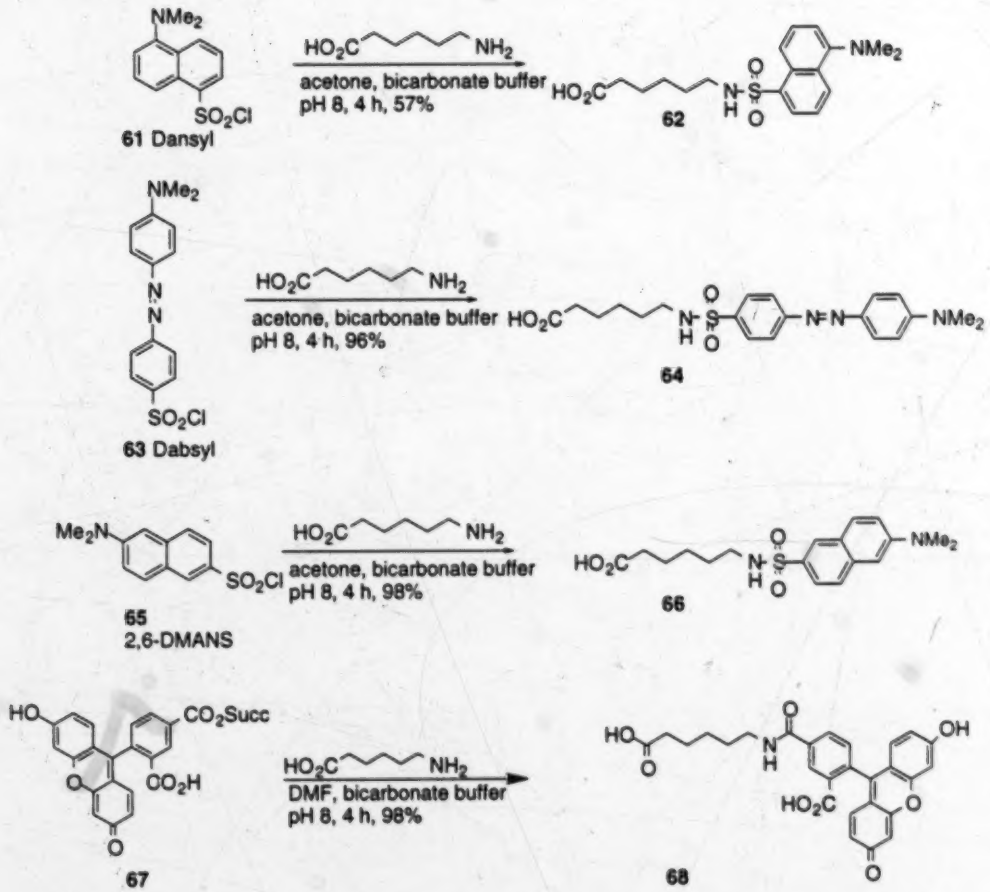
employs trimethylsilyl iodide (TMSI).[44] Compound **59** was subjected to up to 10 equivalents of TMSI, however no deprotection was observed and the starting material was recovered.

Scheme 17



Coupling of the dansyl label to the unprotected amine **60** proved problematic, and only traces of the desired product could be obtained. However, the label can also be added to the spacer before it is coupled to the amine **54**. All of the fluorescent labels were attached to the spacer in a similar manner (Scheme 18).[45] The corresponding acid halides (sulfonyl chlorides), or *N*-hydroxysuccinimide ester in the case of fluorescein, were dissolved in a polar organic solvent and added to a bicarbonate buffer solution (pH 8) containing the dissolved 6-aminocaproic acid to produce the labelled spacer units in good yields.

Scheme 18

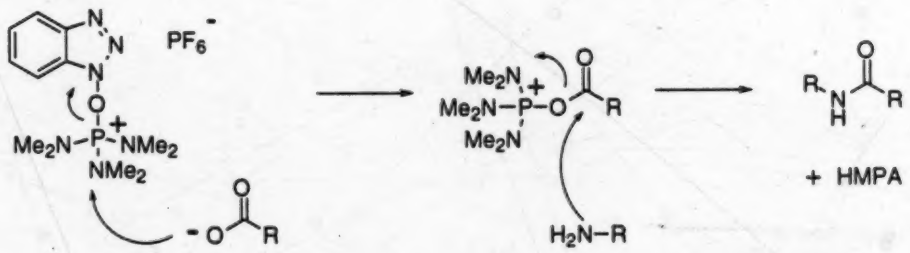


The next step was to couple these labelled spacers to the methyl *para*-amino-phenylbutenoate **54**. Some problems became apparent when this was attempted. The first obstacle is the low nucleophilicity of the amine due to delocalization of the lone pair on the nitrogen into the aromatic ring. The second difficulty is isomerization of the double bond from being conjugated to the aromatic ring to being conjugated to the ester. This problem seems insurmountable given that most common coupling reactions rely on basic conditions to ensure that the nitrogen of the amine is unprotonated and nucleophilic.

The methylene between the aromatic ring and ester is very acidic and treatment with base readily causes some isomerization. The final problem is the stability of the activated spacer, which must withstand the long reaction times necessary to allow the unreactive aniline nitrogen to condense with it.

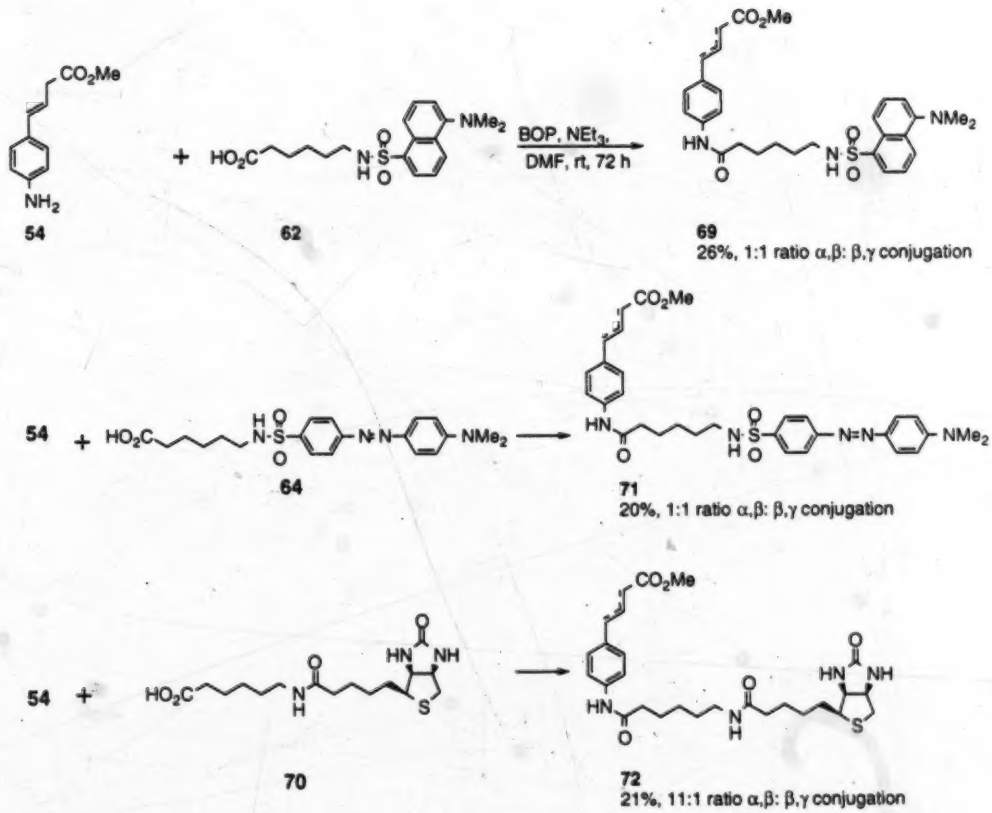
BOP reagent (benzotriazol-2-yloxytris(dimethylamino)phosphonium hexafluoro phosphate) has been successfully used as the coupling agent for the synthesis of hindered peptides that are not sufficiently activated by many other reagents (Figure 10).[30]

Figure 10



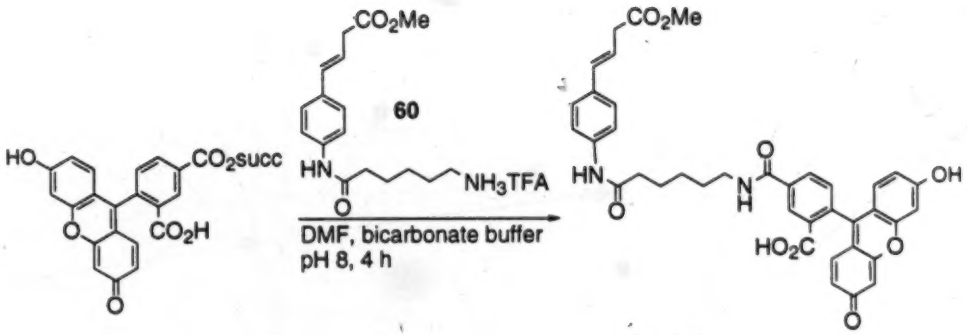
Use of BOP reagent permitted coupling of the dansyl **62**, dabsyl **64** and biotin spacer moieties to the aromatic amine **54** (Scheme 19).

Scheme 19



The coupling of the fluorescein derivative **68** to the aromatic amine **54** failed completely. This is may be due to the presence of a second carboxylic acid on the fluorescein. However, this activated fluorescein derivative could be condensed with **60** using standard conditions[45] to give 84% of the desired product (Scheme 20).

Scheme 20

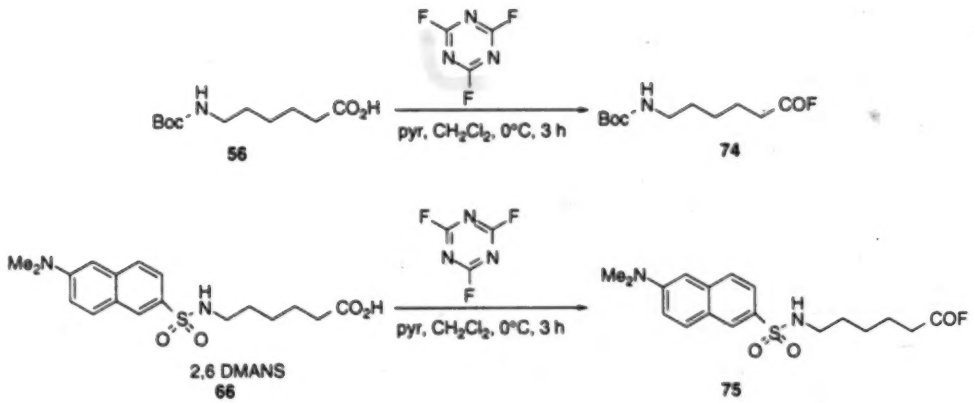


73, 84%

Since the yields of these coupling reactions are still low, a newer coupling method was investigated using the acid fluoride as the activating group.[46] Acid fluorides are much more stable than acid chlorides and should endure the long reaction times necessary for coupling to the unreactive aromatic amine. The disadvantage is that the acid fluorides must be synthesized in a separate step before coupling to the amine (Scheme 21).[46]

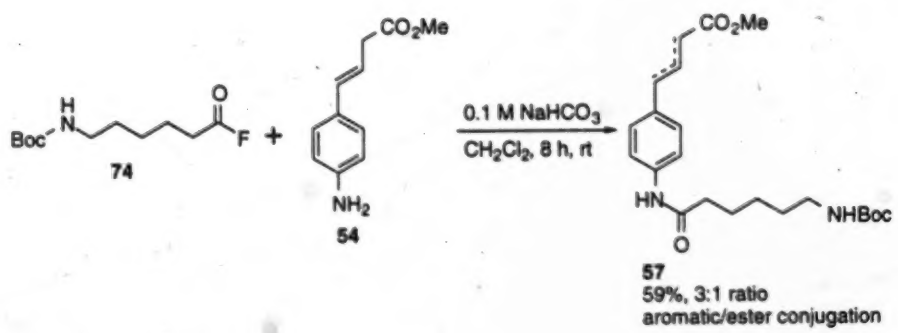
The acid fluoride technique was examined first with the Boc protected spacer and then with the fluorescent label 2,6-DMANS. The Boc derivative **56** or the 2,6-DMANS analogue **66** were stirred with cyanuric fluoride and pyridine. The reaction probably proceeds by a nucleophilic aromatic substitution to yield the acid fluorides **74** (Boc protected) and **75** (2,6-DMANS) in yields of 75 and 81%, respectively.

Scheme 21

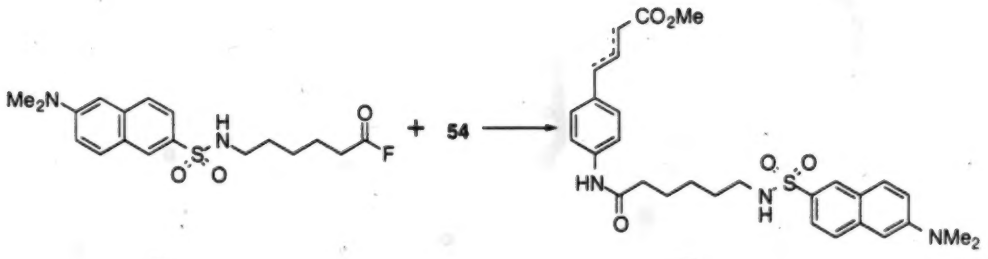


The acid fluorides were coupled to the aromatic amine **54** in a biphasic solution of CH_2Cl_2 and 0.1 M $NaHCO_3$. Although reactive, the acid fluorides can withstand basic aqueous conditions. The reaction times are much shorter than for other coupling reagents (8 versus 72 hours) and the yields are much better (Scheme 22 & 23). However, isomerization of the double bond is still a problem even with the mildly basic conditions (0.1 M $NaHCO_3$).

Scheme 22



Scheme 23



75

76
66%, 5:1 ratio
aromatic/ester conjugation

Comparison of all the coupling reagents used in this project shows that the acyl fluoride is by far superior. It is slightly less convenient in that it must be completed in two steps, but the reaction times are much shorter and the yields much better.

The last step in the synthesis of the labelled inhibitors is the removal of the methyl esters. This hydrolysis is usually completed under basic conditions (i.e. LiOH)[47] that will result in the isomerization of the double bond. However carboxylate ions can be expected to have less tendency to maintain a conjugated double bond than the aromatic ring. Hence the basic conditions could favour the desired β,γ-unsaturated acid products. The methyl esters were deprotected with 2-3 equivalents of LiOH in THF/H_2O (Scheme 24 and Table 6).

Scheme 24

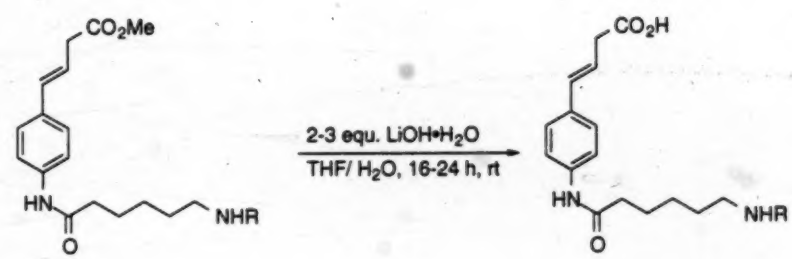


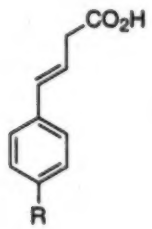
Table 6

Label	Compound	Yield (%)	Ratio aromatic/acid conjugation
Boc	**77**	40	100%
Dansyl	**78**	22	100%
Dabsyl	**79**	8	1/1
Biotin	**80**	8	11/1
Fluorescein	**81**	13	5/1
2,6-DMANS	**82**	13	20/1

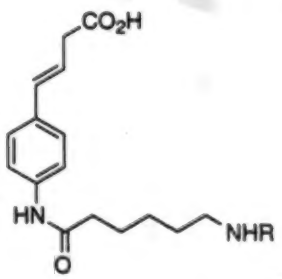
The yields of these methyl ester hydrolyses are low, possibly due to the acidic methylene hydrogens. Deprotonation at this position would result in an anion next to the methyl ester, which would hinder attack by the hydroxide on the carbonyl. Unfortunately, following purification of the free acids by HPLC, some of the undesired double bond isomer was still present in most of the potential inhibitors. These were tested as mixtures to see if any of them were irreversible inactivators of PAM.

Biological Results

A number of the phenylbutenoic acid derivatives were tested against rat PAM by the group of Dr. Betty Eipper at Johns Hopkins University using the same assay conditions. The results are given as IC_{50}'s, (Table 7).



Class I Inhibitors



Class II Inhibitors

Table 7

Class I R=	IC_{50} (μM)	Class II R=	IC_{50} (μM)
H (PBA) **9**	0.4	H **85**	-
NO_2 **83**	8	Boc **77**	20
NH_2 **84**	-	Dabsyl **79**	60
		Fluorescein **81**	60
		2,6-DMANS **82**	50
		Dansyl **78**	0.5
		Biotin **80**	4

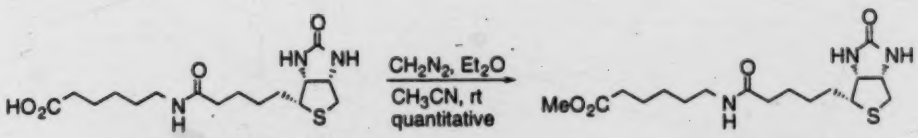
The phenylbutenoic acid analogues that have a positive charge (amino groups) at physiological pH were not inhibitors of PAM. Most of the class II inhibitors are 100 times less effective than the parent phenylbutenoic acid (**9**), except for the dansyl **78** and biotin **80** analogues, which are quite potent. Surprisingly the 2,6-DMANS labelled

inhibitor **82** was much worse than the dansyl analogue **78** despite their being positional isomers which might be expected to have quite similar properties.

Labelled Phenylbutenoic Acids as *In Vivo* Probes of PAM

All of the inhibitors were tested as *in vivo* probes of PAM in cells in tissue culture by Dr. Eipper's group. Unfortunately, none of the fluorescent probes were successful in visualizing PAM in the cell tissue cultures. The biotin derivative **80** resulted in general colourization of the cell when it was linked to a colour reaction *via* an avidin-linked enzyme assay. Since biotin has a biological role of its own,[34b] a test was devised to differentiate general biotin binding from specific binding to PAM. To do this, the biotin-spacer portion (in **80**) was protected as the methyl ester with diazomethane[48] (Scheme 25).

Scheme 25



70 **86**

Examination of **86** with cell tissue cultures gave results identical to biotin labelled PBA **80**, thereby indicating that binding is probably due to the biotin moiety rather than interaction of the phenylbutenoic acid portion with PAM.

Labelled Phenylbutenoic Acids as Mechanistic Probes of PAM

The dansyl labelled inhibitor **78** is a very effective inhibitor of PAM (IC_{50} = 0.5 μM) that is as potent as the parent phenylbutenoic acid (**9**) (IC_{50} = 0.4 μM). Hence it was chosen as a potential mechanistic probe of PAM. PAM (rat medullary thyroid carcinoma, 3 mg) was inhibited with 40 mol equivalents of the dansyl labelled inhibitor (**78**) in the presence of all the necessary cofactors (copper II, oxygen, and ascorbate). It was then digested with endoproteinase Arg-C.[49] This proteolytic enzyme selectively cleaves the peptide on the carboxyl side of arginine residues. The peptide fragments were applied to an HPLC column in 100% Milli Q water with 0.1% TFA and separated by increasing the amount of acetonitrile by 1% per minute to 100% acetonitrile. Dansylated peaks were detected by monitoring the HPLC eluent at 335 nm, (the λ_{max} of the dansyl group). Three peaks were collected, at 18, 27 and 73.5 minutes, (Figure 11).

Figure 11

Unfortunately repeated attempts to analyze these peaks by electrospray mass spectrometry failed to give identifiable signals and thus their composition could not be determined.

The biotin labelled inhibitor **80** was found to be also quite potent, with an IC_{50} only 10 times weaker than the parent phenylbutenoic acid (**9**). A large amount of PAM (3 mg) was inhibited with 10 molar equivalents of **80**. The inhibited enzyme was again digested with endoproteinase Arg-C. The resulting mixture was exposed to immobilized avidin,[50] so that peptides containing the biotin label would bind to the avidin beads allowing the non-labelled peptides to be washed away. The beads were then put on a probe for MALDI TOF mass spectrometry. Three peaks were seen at m/z 1749, 2006 and 2963 (Figure 12).

Figure 12

Examination of the peptide sequence of PAM[20] suggests that these peaks could result from an arginine digest of PAM, once the mass of the inhibitor is removed (Figure 13).

In an attempt to confirm the identity of the peptide fragments, more PAM was inhibited, and the biotin labelled peptides were purified in two steps. The digested peptide fragments were passed through a column that contained avidin immobilized on glass beads. The biotin labelled peptides should bind to the beads and allow the non-labelled peptides to be washed off.[50] Elution of the biotinylated peptides is normally done using guanidine hydrochloride, which denatures the avidin and releases the peptides.[50] The resulting compounds were purified by HPLC, and three peaks were collected with retention times of 49, 51 and 53 minutes. Mass spectrometry of these fractions showed that they were not pure, however masses of 1190 and 992 were recorded (Figure 14).

Figure 13

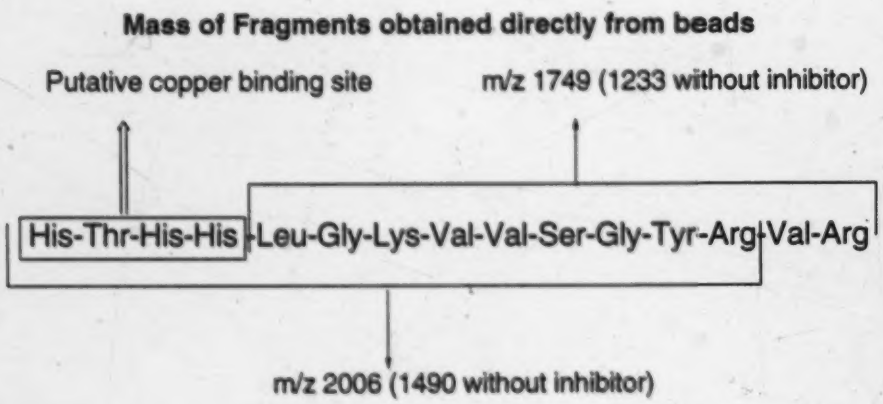
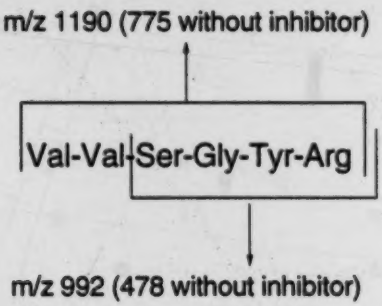


Figure 14

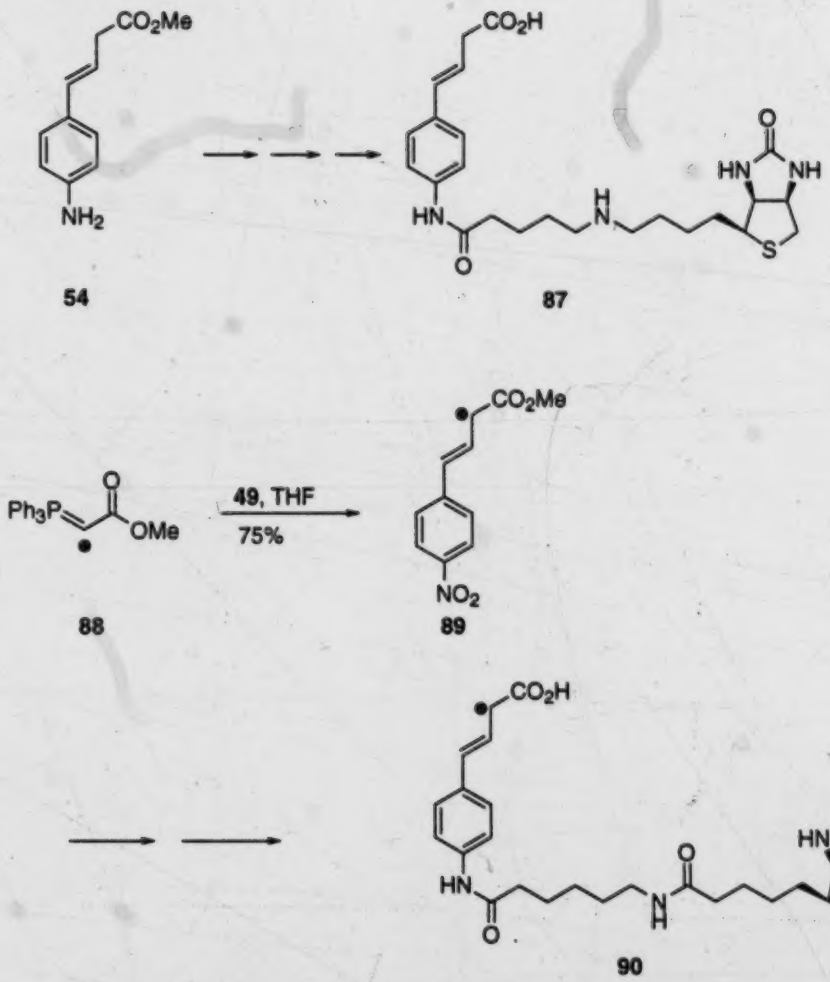
Mass of Fragments obtained from HPLC purification



In an attempt to confirm identity of these fragments, amino acid sequencing (both *C*- and *N*- terminal)[51] was attempted on the HPLC purified fractions. Unfortunately this was not successful, and no sequence could be obtained A different digestion enzyme was used (endoproteinase Asp-N), since this should give peaks with different masses due to cleavage on the *N*-terminal side of aspartic acid residues. Disturbingly, analysis of this digestion gave peaks of the same mass as those derived from the Arg-C cleavage. This suggests that the peaks are artifacts and indicates a fundemental problem with the design of this experiment.

In order to check whether the observed mass spectral peaks contain the biotin derivative, a biotin-labelled inhibitor with a different mass was prepared. This should result in the isolated peptides changing in mass by the difference in mass of the inhibitors. Hence, a biotin-labelled inhibitor with one less carbon in the spacer chain (5 carbons) (**87**) was made in a similar manner (Scheme 26).

Scheme 26



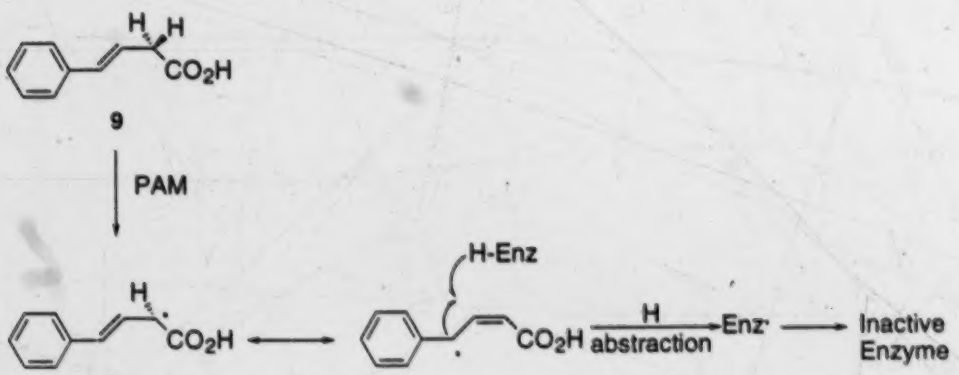
The PAM was then inhibited with compound **87**, in the same manner as before and subjected to MALDI TOF mass spectrometry. The same peaks, with identical masses, were seen as in the digestion of the larger biotin labelled inhibitor **80**. This clearly indicates that the peaks that are being observed are due to non-specific binding of an impurity to the avidin protein on the beads. The peptides do not contain the inhibitor

and the fact that their masses appeared to match masses of peptides near the copper binding site of the enzyme is purely coincidental.

As a last effort to determine by NMR spectrometry whether the inhibitor is covalently attached to PAM the ^{13}C biotin labelled inhibitor **90** was made in the same manner as the non-labelled material that was discussed previously (Scheme 20). The ^{13}C label could in principle be detected by NMR studies of the enzyme or fragments if it is covalently bound.[52] NMR examination of 13 mg of PAM inhibited with the ^{13}C labelled inhibitor **90** did not reveal any direct attachment to PAM or its peptide fragments.[52]

There are several possible conclusions from this study. One is that the inhibitor does covalently bind to the active site of PAM, but the biotin label is cleaved by either the enzymatic digestion or some other workup procedure and is therefore not detected. Another possibility is that the inhibitor is a mechanism based irreversible inhibitor, but it does not itself covalently bind to the active site. For example, the inhibitor could abstract a hydrogen from the active site (i.e. oxidize the enzyme) and thereby cause inactivation (Figure 15). The resulting protein-based radical could either dimerize or undergo electron transfer to Cu (II) to generate a reactive cation. The latter could be quenched by either an internal (protein) or external (i.e. H_2O) nucleophile.

Figure 15



One way to determine whether the inhibitor is covalently bound is to do mass spectral studies on the inhibited enzyme before digestion. Unfortunately, the rat enzyme (MW 75 kDa) is not homogenous and has a mass range of ±2500, due to various post-translational modifications, most likely from differing amounts of glycosylation. It was therefore not possible to do the mass spectral studies directly on the rat enzyme. The honey bee enzyme is homogenous, giving a sharp peak in the MALDI mass spectrum. It is an ideal candidate for these studies. However, it must be sequenced to determine the site of inactivation. Studies are currently underway to sequence the bee PHM and to use it to determine if any covalent modification occurs using the biotin labelled phenylbutenoic acid (**80**).

Substituted Phenylbutenoic Acids as Inhibitors of PAM

A structure activity study of some inhibitors of PAM has been previously reported (Table 3),[22] however, only the parent compound of the phenylbutenoic acids, was included in the study.

Table 3: Structure Activity Relationships Among Inhibitors of PAM

Organic Acid	50% Competition (mmol)	50% Inactivation (mmol)	C_{50}/I_{50}
2-Pentenoic acid	74.98	20.96	3.58
2-Hexenoic acid	46.46	28.13	1.65
2,4-Hexadienoic acid	11.09	34.99	0.32
2,4,6-Octatrienoic acid	5.67	3.86	1.47
Vinylacetic acid	13.06	6.36	2.05
4-Phenyl-3-butenoic acid	0.031	0.011	2.82
Phenylpropiolic acid	48.62	39.92	1.22

The goal of this section of the project was to synthesize a variety of substituted phenylbutenoic acids and test them as inhibitors of PAM. This inhibition data may allow some conclusions to be made about the spatial requirements in the active site and whether or not electron withdrawing or electron donating substituents are favoured. A phenylbutenoic acid derivative in which the hydrogens of the methylene carbon have been substituted for deuterium was also synthesized and used to determine if there is any isotope effect during the inhibition of PAM.

These potential inhibitors will be tested on both rat PAM[11] and bee PHM[11], in order to determine whether specificity for the insect or mammalian enzyme can be achieved. Such differences can potentially be exploited to create insecticides because many important insect neuropeptides possess *C*-terminal amides.

Synthesis of Substituted Phenylbutenoic Acids

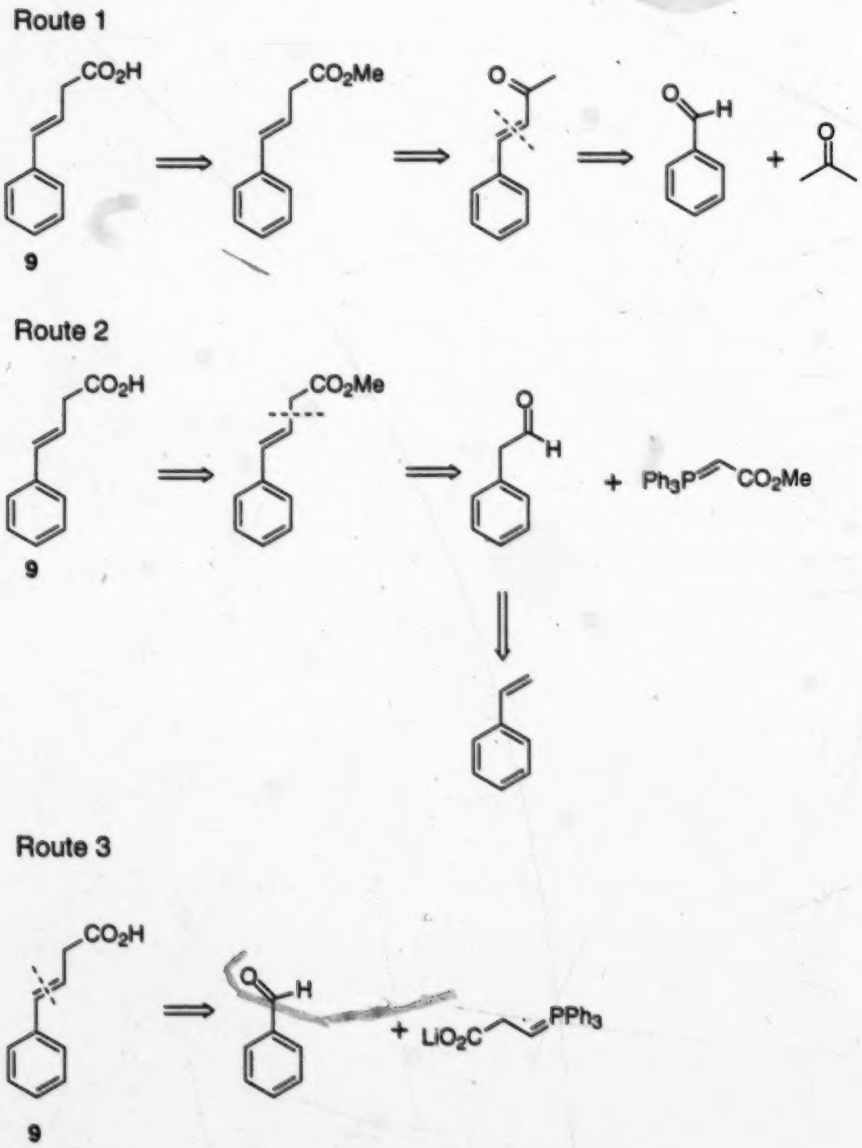
A useful synthetic route would allow for simple aromatic starting materials that are available with a wide variety of substituents on the aromatic ring. Three possible routes have been examined that lead to substituted phenylbutenoic acids (Figure 16).

Route 1

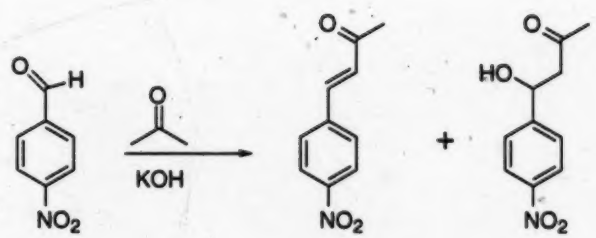
The main advantage of this route is that it starts from benzaldehyde, and many substituted benzaldehydes are commercially available. This approach was investigated using *para*-nitrobenzaldehyde as a starting material, (Scheme 27).

Acetone provided an inexpensive source of the required 3 carbons, *para*-nitrobenzaldehyde undergoes an aldol reaction with acetone using potassium hydroxide as a base to give a small amount of the desired alkene **91,** but produces primarily the alcohol **92** which has not undergone elimination.[53] The alcohol **92** could be converted to the alkene **91** by dehydration. A solution of **92** in benzene and a small amount of *para*-toluenesulfonic acid was heated to reflux and the water that was eliminated was absorbed by a Soxhlet extractor containing CaH_2. Either the purified alcohol **92** or the mixture of alcohol **92** and alkene **91** obtained directly from the aldol reaction could be used for the dehydration (Scheme 28).

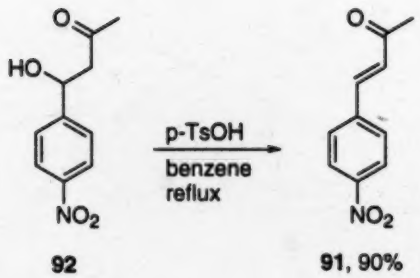
Figure 16: Retrosynthetic Analysis of Synthetic Routes to PBA



Scheme 27

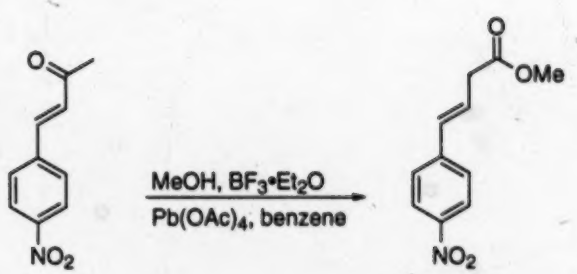


Scheme 28



Rearrangement of the unsaturated ketone gives the methyl ester of the substituted phenylbutenoic acid.[54] Typically, the unsaturated ketone, 3 equivalents of methanol and $BF_3 \cdot Et_2O$ (0.8 equivalent) are dissolved in benzene and added to a suspension of lead tetraacetate (1.5 equivalents) (Scheme 29). The methyl ester **50** is difficult to purify and tends to polymerize and decompose on silica gel during chromatography. It can be purified using the less acidic Florisil for column chromatography.

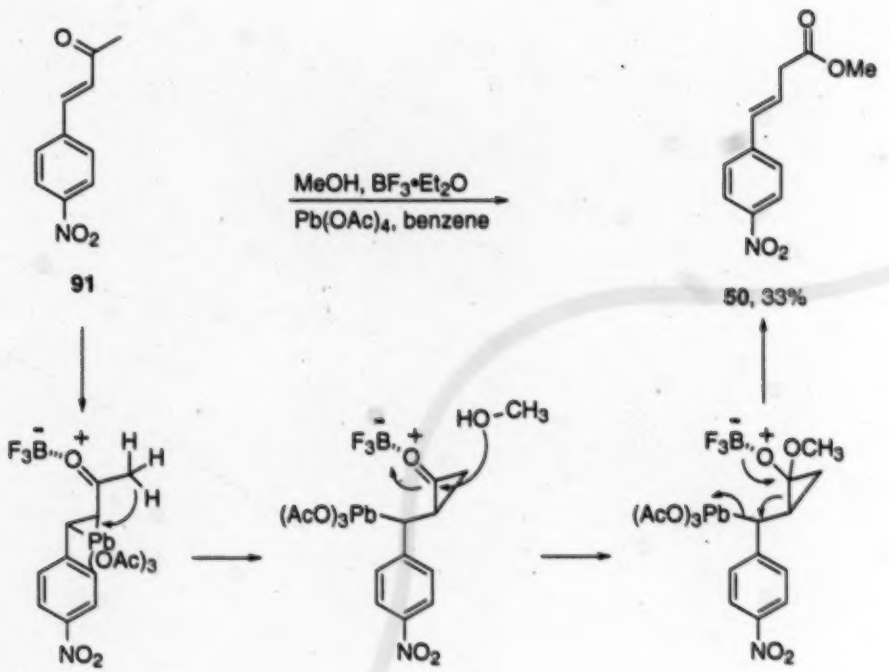
Scheme 29



91 **50, 33%**

The mechanism of this reaction is not known but may proceed as shown in Figure 17. Activation of the double bond and the ketone with the lead tetraacetate and $BF_3 \cdot Et_2O$, respectively, would greatly increase the acidity of the protons of the methyl group alpha to the ketone. Loss of one of these protons could allow formation of the cyclopropanone. Nucleophilic attack by the methanol on the ketone, followed by opening of the cyclopropyl ring leads to the target methyl ester of phenylbutenoic acid (**50**). The yield for this reaction is quite low; the best yield obtained is 33% and is frequently in the area of only 10-15%. This low yield coupled with a difficult purification made this reaction unattractive as the key step in a route to a variety of substituted phenylbutenoic acids.

Figure 17

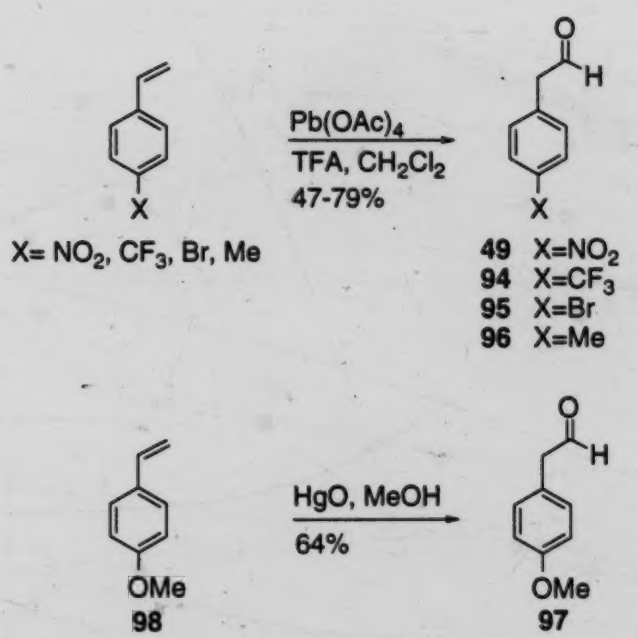


Route 2

The second route uses styrene as a starting material for the synthesis. Substituted styrenes are not as readily available as benzaldehydes but several can be obtained commercially. Five *para*-substituted styrenes with groups ranging from electron donating (MeO, Me) to electron withdrawing (NO_2, CF_3, Br) were used to test the feasibility of this route.

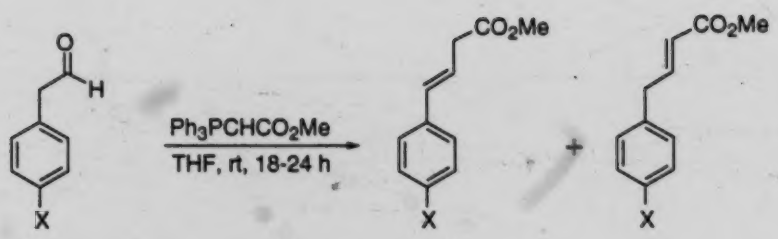
Styrenes can be oxidized to the phenylacetaldehydes **49, 94-96** using lead tetraacetate and trifluoroacetic acid, with yields ranging from 47-79%.[35] The *para*-methoxystyrene is best oxidized with mercuric oxide in methanol to yield the corresponding *para*-methoxyphenylacetaldehyde **97** in 64% yield (Scheme 30).[55] The yields of these oxidations are only moderate, and the sensitive aldehydes were not completely purified but used immediately in the next reaction.

Scheme 30



The remaining two carbon atoms can be easily added to the aldehyde *via* a Wittig reaction. The Wittig reagent chosen was (carboxymethylmethylene)triphenylphosphorane, which is stabilized and should give primarily the desired *trans* product.[36] The reaction was found to give a mixture of α,β and β,γ double bond isomers whose ratio is somewhat dependent on the *para*-substituent of the aromatic ring. The double bond can be conjugated to the ester (α,β) or the aromatic ring (β,γ), and addition of excess Wittig reagent (a base) aids in the isomerization to the desired β,γ ester. As expected, electron withdrawing *para*-substituents on the aromatic ring favour conjugation to the aromatic ring. In the case of the *para*-nitro aldehyde **49**, only the β,γ conjugated ester isomer can be detected (Table 8). The yields from the Wittig reaction range from good (80-89%) with the electron withdrawing substituents (NO_2, **50**; Br, **99**) to only moderate (34-37%) with the electron donating substituents (Me, **100**; MeO, **101**), the exception being the CF_3 analogue **102** which affords only a 30% yield (Scheme 31).

Scheme 31



X= NO_2, CF_3, Br, Me, MeO

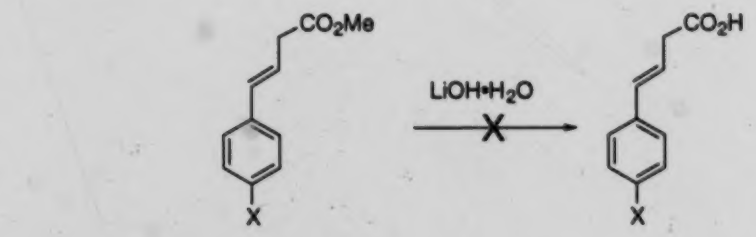
Table 8

Para Substituent	Compound Number	Yield	Ratio aromatic/ester conjugation
NO_2	**50**	80%	100% aromatic
CF_3	**102**	30%	7/1
Br	**99**	89%	4/1
Me	**100**	34%	1/1
MeO	**101**	37%	1/4

The double bond isomers were not separated, because it seemed likely that treatment with LiOH for removal of the methyl esters would isomerize the double bond, hopefully into conjugation with the aromatic ring in preference to the carboxylate ion. Although such methyl esters are commonly hydrolysed with lithium hydroxide,[47] this proved to be very problematic in some cases. Any attempt to deprotect the more electron withdrawing analogues (NO_2 **50**, CF_3 **102**) gives no free acid and results in a complex mixture possibly due to polymerization. The other analogues were successfully

deprotected to give the free acids (Br, **103**; Me, **104**; MeO, **105**), albeit in very low yields. Unfortunately, the isomerization of the double bond into conjugation with the aromatic ring did not proceed as desired, and isomeric mixtures were obtained (Scheme 32, Table 9).

Scheme 32



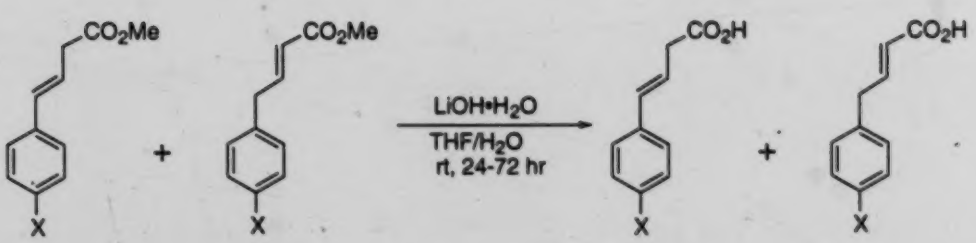
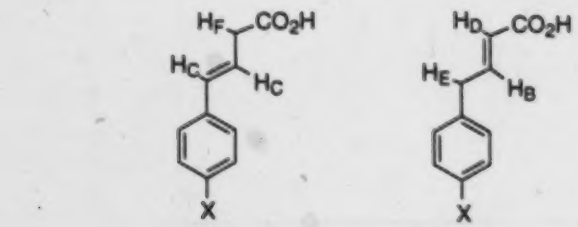


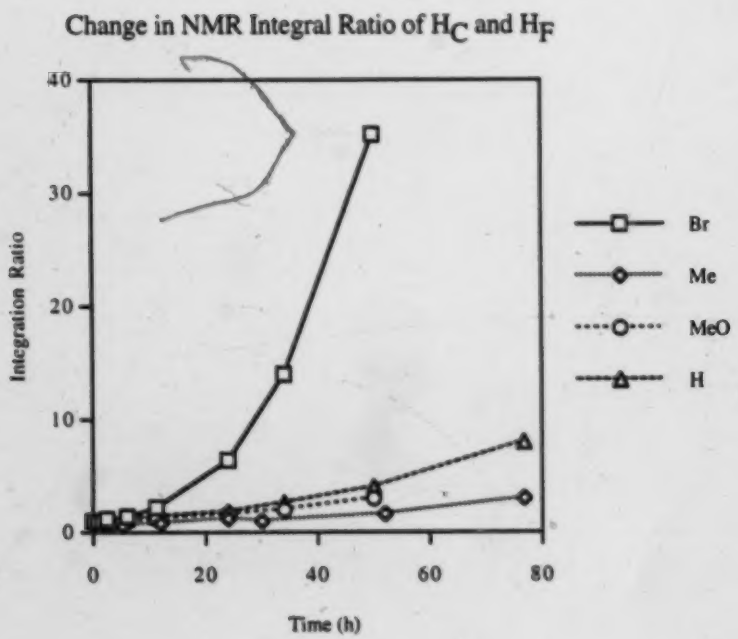
Table 9

Para Substituent	Compound	Yield	Ratio aromatic/acid conjugation
Br	**103**	13%	1/1
CH_3	**104**	16%	1/1
CH_3O	**105**	11%	1/4

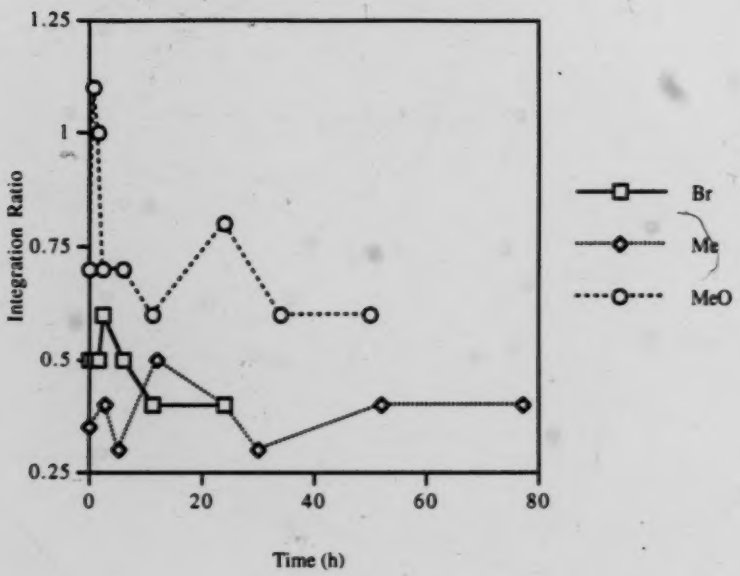
In an attempt to isomerize the double bond into conjugation with the aromatic ring, the acids (Br, **103**; Me, **104**; MeO, **105**) were dissolved in D_2O and 3 equivalents of LiOH were added. The isomerization was followed by NMR spectrometry by integrating the peak areas for the olefinic and allylic resonances, and the results are shown in Figure 58

Figure 58

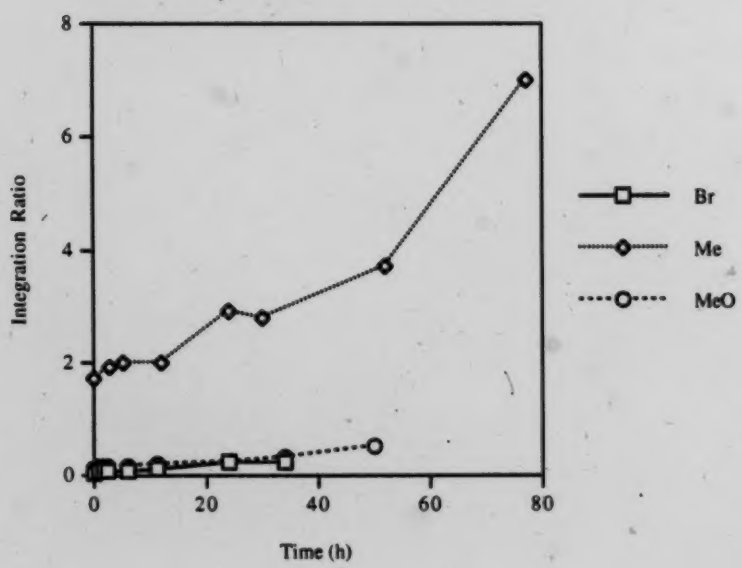




Change in NMR Integral Ratio of H_D and H_E



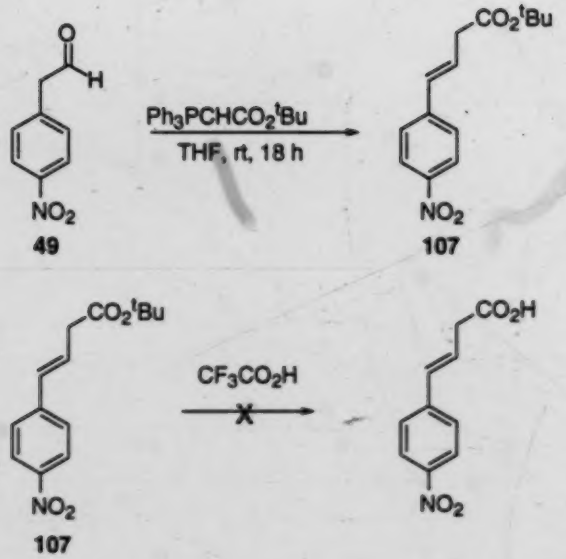
Change in NMR Integral Ratio of H_E and H_F



The isomerization occurred slowly, but a small amount of the isomer in which the double bond was conjugated with the carboxylate group always remained. A few other conclusions can be made about the NMR study. The ratio of C/F slowly increases with time. This indicates that the hydrogens on the methylene carbon are being exchanged with deuterium in the isomer in which the double bond is conjugated to the aromatic ring. The ratio of D/E is reasonably stable, indicating that the methylene hydrogens of the other isomer are not exchanging for deuterium. The ratio of E/F is increasing with time. At first glance this might mean that the isomerization is occuring towards the isomer in which the double bond is conjugated to the carboxylate, however this is complicated by the deuterium exchange occuring in only one isomer. As the double bond shifts to being in conjugation with the aromatic ring, it is exchanged for deuterium. Since the isomerization is not occuring to any great extent in the opposite direction, deuterium exchange does not occur at that methylene and the ratio of E/F therefore gets larger with time. The deuterium exchange at the methylene carbon was utilized to synthesize *trans*-2,2-dideutero-4-phenyl-3-butenoic acid (**106**) in quantitative yield.

The pure *trans*-3-butenoic acids **103**, **104**, **105** can be obtained by recrystallization from ethyl acetate/hexane once the majority of the unwanted double bond isomer is lost by treatment with base. The methyl ester of the *para*-nitro and *para*-trifluoromethyl analogues could not be removed without decomposing or polymerizing the material. A variety of other methods were attempted, including TMSI, to BBr_3.[56] Since hydrolysis of the methyl esters was unsuccessful, the *tert*-butyl ester of the *para*-nitro-phenylbutenoic acid was prepared from the aldehyde by a similar route[36] to give **107** in 51% yield. The deprotection of this ester with trifluoracetic acid[57] was also unsuccessful and gave only decomposition, (Scheme 33).

Scheme 33

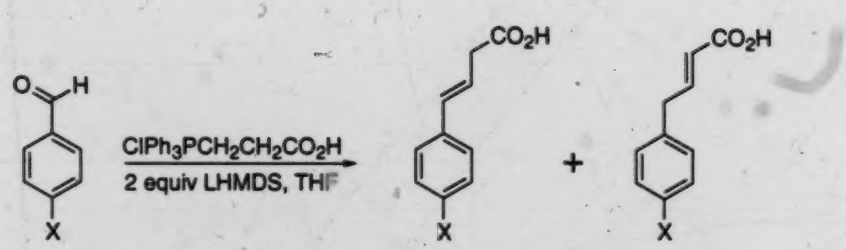


The problems with the deprotection of the methyl esters made this approach to a wide variety of phenylbutenoic acids undesirable.

Route 3

The last route investigated returned to using the readily available benzaldehydes as starting materials, and added the necessary 3 carbons with an unstabilized Wittig reagent derived from 3-chloropropionic acid (Scheme 34).[58]

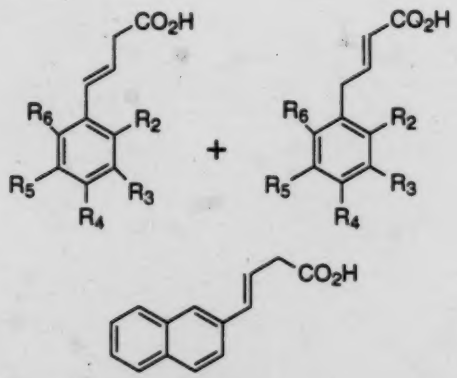
Scheme 34



The dianion of the Wittig reagent is required as the carboxylic acid will be deprotonated by the base before the methylene hydrogen. The original paper by Corey *et al.*[58] utilized NaH as a base; however, LHMDS was found to be a superior base for forming the dianion and completing the reaction. The precursor to the Wittig reagent, **108** was easily made by heating triphenylphosphine and 3-chloropropionic acid. It was less easily recrystallized as a glass formed during the reaction that was difficult to dissolve. It could however be made in large quantities (47 g) in a reasonable yield (67%). The orange dianion of this reagent was formed with LHMDS and was then added to benzaldehyde. The yields of these reactions utilizing a wide variety of benzaldehydes were moderate to good (37-72%), but this "one pot synthesis" of the phenylbutenoic acids renders them acceptable. As expected, isomerization of the double bond is a problem with these analogues, and they were therefore purified by HPLC. The *para*-nitro analogue could not be synthesized this way as it decomposed, however a wide variety of other *para*-substituents were prepared, both electron withdrawing and electron donating (Table 10).

The placement of the substituent on the aromatic ring could also be important, and several *ortho*- and *meta*- benzaldehydes were used, as were several disubstituted benzaldehydes and a naphthalene analogue. Acetophenone was also an acceptable substrate for this reaction and yielded the methyl substituted double bond analogue **109** in a moderate yield (36%), but as a 15/1 ratio of *E/Z* isomers. Although the problem of double bond isomerism has not been resolved, this is approach offers the most facile access to

phenylbutenoic acids. It has only one step, gives reasonable yields, and the products can be easily purified by HPLC.



122

Table 10

Compound	Substituent	Position R_x	Yield (%)	Ratio aromatic/acid conjugation
9	H	-	68	-
110	OMe	2,4	60	98/2
111	Cl	4	72	95/5
112	Cl	2	65	9/1
113	CN	4	70	1/1
114	CF_3	4	46	9/1
115	CF_3	2	37	9/1
116	CF_3	3	42	8/1
117	Cl	3	64	10/1
118	OMe	3	43	6/1
119	Cl	3,4	49	15/1
120	Cl	2,6	40	15/1
121	Cl	2,6	12	1/9
122	naphthalene	2	72	100%

Summary

The study of the tripeptide styrylglycine derivatives indicates that the double bond in PBA **9** is mimicking the amide bond in the natural peptide substrates. Attempts to determine whether phenylbutenoic acids are covalently attached to PAM during the inactivation process were inconclusive. It could be that the methodology is cleaving the inhibitors from the peptide or that the PBA derivatives do not covalently bind to the active site, but instead set off a chain of events that inactivates the enzyme. Mass spectrometric studies on homogeneous enzyme may allow this question to be resolved. Three approaches to synthesis of simple substituted phenylbutenoic acids were explored. The simplest and most effective route involves direct condensation of the Wittig reagent derived from 2-carboxyethyl triphenylphosphonium chloride with the corresponding benzaldehydes. Like the other routes, this approach often gives a mixture of α,β and β,γ isomers that are difficult to separate. However, ongoing inhibition studies with both rat PAM and bee PHM may shed more light on the electronic requirements of the active site. These results together with the present synthetic routes should allow improved and selective inhibitors to be designed.

Experimental

General

All non-aqueous reactions requiring anhydrous conditions were performed under a positive pressure of argon (Ar) in oven dried glassware, which had been allowed to cool under Ar. All solvents for anhydrous reactions were dried according to Perrin *et al.*[59] Tetrahydrofuran, diethyl ether, benzene and toluene were distilled from sodium and benzophenone. Triethylamine, pyridine, chloroform, dichloromethane and acetonitrile were distilled from calcium hydride. Anhydrous methanol was purified by distillation from magnesium metal and catalytic iodine. The removal of solvents refers to evaporation *in vacuo* on a rotary evaporator followed by evacuation to constant sample weight (<0.1 mm Hg). Solvents used for chromatography that were not reagent grade were distilled prior to use. Water was obtained from a Milli-Q reagent water system. Buffers were prepared with Milli-Q water and the pH was adjusted to the desired value at room temperature with NaOH and HCl solutions.

All reagents employed were of American Chemical Society (ACS) grade or finer. All commercially available isotopically labelled compounds were purchased from Cambridge Isotope Laboratories (Andover, MA). Commercial and organometallic reagents were obtained from Aldrich Chemical Co. (Milwaukee, WI) unless otherwise specified. n-Butyllithium solution was periodically titrated against methanol/phenanthroline.[60] Amino acids and amino acid derivatives used as starting materials were obtained from Sigma Chemical Co. Commercially available enzymes were obtained from Sigma. Catalase (from bovine liver) was purchased as an aseptically filtered aqueous solution from Sigma. Fast flow Sepharose was obtained from Pharmacia Labs Inc. (Piscataway, NJ, USA). Affigel 15 was obtained from Biorad (Mississauga, ON). Rat PAM (peptidylglycine α-amidating monooxygenase) was a generous gift of Unigene Inc. (New Jersey) and Dr. D. Merkler (Department of Biochemistry, Duquesne University).

Whenever possible reactions were followed by thin layer chromatography (TLC) and visualized using UV, fluorescence, iodine staining, dodecamolybdophosphoric acid, and/or ninhydrin. Commercial thin layer chromatography plates were normal silica (Merck 60 F-254) or reverse-phase (Merck RP-8 or RP-18 F-254S). Flash chromatography was performed according to Still *et al.* using Merck type 60, 230-420 mesh silica gel.[61] Florisil for column chromatography was Fisher Type F100-500, 60-100 mesh.

High pressure liquid chromatography (HPLC) was performed on: 1) a Beckman System Gold instrument equipped with a model 166 variable wavelength UV detector and an Altex 210A injector with 100 µL sample loop; 2) a BioRad Gradient Module fitted with a variable wavelength detector and a 100 µL sample loop or 3) a Rainin Dynamax SD-200 pumps with a Rainin Dynamax UV-1 variable length detector fitted with a 5 mL sample loop. The columns were Waters Nova-Pak (reverse phase 8NVC18 4µ C_{18}), µBondapak (reverse phase 8MBC1810µ) and Resolve (reverse phase 25x100 mm C18, 90Å). A Vydac 218TP54 steel column was used in the enzyme digestion purification studies. All HPLC solvents were prepared fresh daily and filtered with a Millipore filtration system before use.

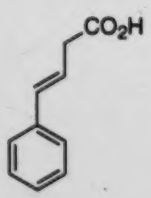
All literature compounds had IR, ^{1}H NMR and mass spectra consistent with the assigned structures. Melting points are uncorrected and were determined on a Thomas Hoover or Büchi oil immersion apparatus using open capillary tubes. Optical rotations were measured on Perkin Elmer 241 or 141 polarimeter with a microcell (100 mm, 1 mL) at ambient temperature. All specific rotations reported were measured at the sodium D line and values quoted are valid within ±1°. Infrared spectra (IR) were recorded on Nicolet 7199 or 20SX FT-IR spectrometer. Unless otherwise stated, mass spectra (MS) were recorded on Kratos AEI MS-50 (high resolution, electron impact ionization), MS-12 (chemical ionization, CI, NH_3) and MS-9 (fast atom bombardment with argon, posFAB) instruments. Cleland matrix in posFAB spectra refers to a 5:1 mixture of dithiothreitol and

dithioerythritol. Microanalyses were obtained using Perkin Elmer 240 or Carlo Erba 1108 CHN analyzers.

Nuclear magnetic resonance (NMR) spectra were measured on Bruker WH-200, AM-300, WM-400, or Varian 500 instruments in the specified solvent, and spectra were referenced to the residual solvent peak for ^{1}H NMR. For ^{13}C NMR spectra, the deuterated solvent peak was used as the reference with its position set relative to TMS. For ^{19}F NMR spectra, $CFCl_3$ was added and used as the internal reference. The composition of isomeric ratios was determined by integratation.

Honey bees were a generous gift of Cor Dewitt of Edmonton, Alberta, and were stored frozen at -70°C until needed. Homogenization was performed using an IKA-Werk Ultra-Turrax homogenizer. A Sorvall RC-5B refrigerated superspeed centrifuge was used in the enzyme isolation. An LKB peristaltic pump was employed to control the flow rate on Sepharose and Affigel columns.

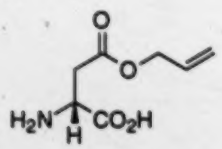
_E_-4-Phenyl-3-butenoic Acid (9).[58]



The literature procedure was modified.[58] Hexamethyldisilazane (4.0 mL, 19 mmol) was dissolved in THF (50 mL) and the solution was cooled to 0°C with stirring under Ar. BuLi (11 mL, 1.8 M, 19 mmol) was added and the solution was stirred for 15 minutes. The solution was cooled to -78°C and compound **79** (3.2 g, 9.5 mmol) was added. The reaction mixture was allowed to warm to -30°C and stirred for 45 minutes. The solution was then recooled to -78°C, benzaldehyde (1.0 g, 9.4 mmol) was added and the mixture was allowed to warm to room temperature and stirred for 16 hours. The mixture was

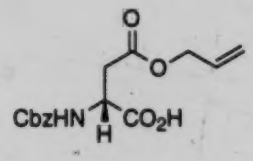
diluted with H_2O (150 mL) and washed with CH_2Cl_2 (50 mL). The aqueous phase was acidified to pH 2 with HCl (concentrated) and extracted with CH_2Cl_2 (3x100 mL). The solvent was dried ($MgSO_4$) and removed, and the residue was purified by chromatography (Florisil, 25% Et_2O in petroleum ether to 100% Et_2O, then 10% MeOH in Et_2O, R_f 0.3 in Et_2O) to give the product **9** (0.95 g, 68%): mp 83-85 °C (lit.[58] 84-86 °C) IR ($CHCl_3$ cast), 3071 (s, br), 2980 (s), 2726 (m), 1563 (m), 1686 (s), 1618 (m), 1584 (m), 1453 (s), 1424 (s), 1326 (s), 1292 (s), 935 (s), 709 (s) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 12.2 (br, s, 1H, $CO_2\underline{H}$), 7.5 (m, 5H, aryl-C$\underline{H}$), 6.6 (d, J = 16.2 Hz, 1H, C$\underline{H}$=CHCH$_2$), 6.4 (d of t, J = 16.2, 7.6Hz, 1H, CH=C$\underline{H}$CH$_2$), 3.3 (d, J = 7.6 Hz, 2H, C$\underline{H}_2$); MS calcd for $C_{10}H_{10}O_2$, 162.0681; found 162.0684

β-Allyl-D-aspartate (14).[72]



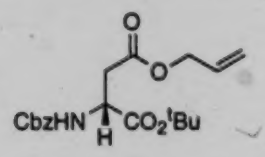
D-Aspartic acid (5.0 g, 37 mmol) was placed in a round bottom flask and allyl alcohol (50 mL, 1.2 mol) was added followed by TMSCl (9.4 mL, 74 mmol). The mixture was stirred for 16 hours, poured into Et_2O (200 mL) and filtered to give the product **14** (5.6 g, 85%): mp 181-182°C; IR (μscope) 3250-2400 (br s), 1740 (s), 1725 (s) cm^{-1}; ^1H NMR (300 MHz, D_2O) δ 6.0 (d of d of d of d, J = 19.6, 10.5, 5.8, 3 Hz, 1H, CH$_2$C$\underline{H}$=), 5.3 (d of q, J = 10.5, 1.5 Hz, 1H, C$\underline{H}$= (cis)), 5.3 (d of q, J = 19.6, 1.5 Hz, 1 H, C$\underline{H}$= (trans)), 4.7 (d of d, J = 4.6, 3.0 Hz, 2H, C$\underline{H}_2$-allyl ester), 4.4 (d of t, J = 5.8, 2 Hz, 1H, α-C$\underline{H}$), 3.2 (m, 2H, β-C$\underline{H}_2$); MS (posFAB) $C_7H_{11}NO_4$, 173; found 174 (MH^+, 100%).

β-Allyl-*N*-Cbz-D-aspartate (15).[72]



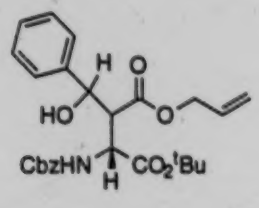
The allyl protected D-aspartate **14** (6.5 g, 37 mmol) was dissolved in H_2O (100 mL) and Et_2O (50 mL) was added. Potassium carbonate (6.2 g, 44 mmol) and benzyl chloroformate (5.6 g, 0.32 mmol) were added and the biphasic solution was stirred for 4 hours. The layers were separated and the aqueous phase was washed with Et_2O (2x50 mL), acidified to pH 1 with 2M HCl and then extracted with Et_2O (3x50 mL). The organic extracts were dried ($MgSO_4$) and the solvent was removed. The residue was further purified by flash chromatography (SiO_2, 25% Et_2O in petroleum ether to 100% Et_2O, R_f 0.3 (25% Et_2O) to give the product **15** (6.3 g, 72%) as an oil: IR (CH_2Cl_2 cast) 3650-2400 (br m), 1735 (s) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 10.3 (s br, 1H, COOH), 7.3 (m, 5H, aryl-H), 6.0 (d, J = 8.5 Hz, 1H, NH), 5.9 (d of d of t, J = 17.1, 10.5, 5.8 Hz, 1H, CH_2CH=), 5.3 (d of d, J = 17.1, 1.3 Hz, 1H, CH= (trans)), 5.3 (d of d, J =10.5, 1.3 Hz, 1H, CH=(cis)), 5.0 (s, 2H, CH_2 benzyl ester), 4.7 (m, 1H α-CH), 4.6 (d, J = 5.2 Hz, 2H, CH_2 allyl ester), 3.1 (d of d J = 17.3, 4.6 Hz, 1H, β-CH), 3.0 (d of d J = 17.1, 4.6 Hz, 1H, β-CH); MS (EI) calcd for $C_{15}H_{17}NO_6$, 307.1056; found 307.1062 (5%), 91 (100%).

β-Allyl-α-*tert*-butyl-*N*-Cbz-D-aspartate (16).[72]



The diprotected aspartate **15** (5.3 g, 17 mmol) was dissolved in CH_2Cl_2 (75 mL) and concentrated H_2SO_4 (0.5 mL) was added. Isobutene was bubbled through the solution for 45 minutes and the mixture was stirred for 48 hours. It was then poured into $NaHCO_3$ (saturated, 100 mL) and the organic phase was separated. The aqueous phase was washed with CH_2Cl_2 (3x50 mL), the organic phases were combined, dried ($MgSO_4$) and the solvent was removed. The residue was further purified by flash chromatography (SiO_2, 10% Et_2O in petroleum ether to 100% Et_2O, R_f: 0.7 in 50% Et_2O) to give the fully protected amino acid **16** (4.3 g, 68%) as an oil: $[\alpha]_D$= -20.2; IR ($CHCl_3$ cast) 3355 (br, m), 2980 (m), 1730 (s) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.3 (m, 5H, aryl-H), 6.0 (d, J = 7.9 Hz, 1H, NH), 5.9 (d of d of t, J = 17.2, 10.5, 5.7 Hz, 1H, CH_2CH=), 5.1 (s, 2H, CH_2 benzyl ester), 4.9 (d of d, J =10.5, 1.3 Hz, 1H, CH=(cis)), 4.9 (d of d, J = 17.2, 1.3 Hz, 1H, CH=(trans)), 4.3 (m, 1H, α-CH), 4.2 (d, J = 5.2 Hz, 2H, CH_2 allyl ester), 3.0 (d of d J = 16.7, 4.5 Hz, 1H, β-CH), 2.7 (d of d J = 16.7, 4.5 Hz, 1H, β-CH), 1.3 (s, 9H, $(CH_3)_3C$); MS (FAB) $C_{19}H_{25}NO_6$; 364 (MH^+, 1%), 91 (100%).

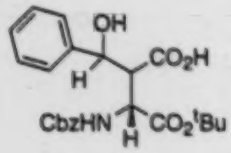
(2R)-3-Allyloxycarbonyl-2-benzyloxycarbonylamino-4-hydroxy-4-phenyl-butanoic Acid *tert* -Butyl Ester (17)[72].



Freshly distilled HMDS (8.4 mL, 73 mmol) was dissolved in THF (70 mL), the solution was stirred under Ar, cooled to 0°C and BuLi (19 mL, 1.1 M, 22 mmol) was added. It was stirred for a further 10 minutes and then cooled to -78°C. The fully protected aspartate **16** (3.5 g, 11 mmol) was dissolved in THF (40 mL) and added to the LHMDS solution, the temperature was allowed to rise to -30°C and the solution was stirred for 45 minutes before being recooled to -78°C. Benzaldehyde (3.5 mL, 30 mmol) was added and the mixture was stirred a final 15 minutes. It was then poured into 1M HCl (100 mL) and then extracted with Et_2O (3x100 mL). The organic phase was dried ($MgSO_4$) and the solvent removed. The residue was purified by flash chromatography (SiO_2, 20% Et_2O in petroleum ether to 100% Et_2O, R_f 0.30 (Et_2O:hexane, 2:3) to give the product **17** as an oil (3.2 g 83%) as a 4:1 mixture of isomers at the aldol addition site (C-4). The product an oil: $[\alpha]_D$ = -1.6 (c=0.92 in $CHCl_3$); IR ($CHCl_3$ cast) 3420 (br), 2979 (m), 1729 (s), 1601 (s) 1455 (m), 1398 (m), 1369 (m), 1342 (m), 1221 (m), 1155 (m), 1060 (m), 1028 (w), 1001 (w), 988 (m), 749 (s), 699 (s) cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) δ 7.4 (m, 10H, aryl-H), 5.9 (m, 1H, CH=), 5.1 (m, 6.8H, CH_2 benzyl ester, CH_2 allyl ester, CH_2=, CHOH (major isomer)), 4.6 (m, 2.2H, α-CH, CHOH (minor isomer), OH), 4.2 (m, 0.2H, α-CH minor isomer), 4.0 (s, br, 0.2H, 3.9, NH minor isomer), 3.4 (m, 1H, β-CH), 3.2 (s, br, 0.8H, NH major isomer) 1.6 (s, 1.8H, $(CH_3)_3C$ minor isomer), 1.5 (s, 7.2H, $(CH_3)_3C$ major isomer); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 171.9, 169.1, 155.8, 140.3, 136.2,

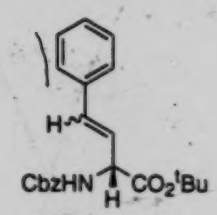
131.3, 128.5, 128.3, 128.0, 126.5, 119.0, 82.6, 72.6, 66.8, 66.0, 53.5, 28.0, 27.6; MS (FAB) $C_{26}H_{29}NO_7$; 470 (MH^+, 2.5%), 91 (100%).

(2*R*)-2-Benzyloxycarbonylamino-3-carboxy-4-hydroxy-4-phenyl butanoic Acid *tert*-Butyl Ester (18)[72].



Compound **17** (2.9 g, 6.2 mmol) was dissolved in 25 mL of degassed CH_2Cl_2. Pyrrolidine (0.78 mL, 94 mmol), triphenylphosphine (94 mg, 0.36 mmol) and $Pd(PPh_3)_4$ (205 mg, 0.20 mmol) were added, and the mixture was stirred under Ar at room temperature for 10 minutes. The solution was then washed with 1 M HCl (3x20 mL), dried with Na_2SO_4 and the solvent was removed. The crude product was purified by flash chromatography (SiO_2, Et_2O/CH_2Cl_2/TFA: 30/70/0.1, R_f 0.43) to give **18** (2.26 g, 86%) as an oil: IR ($CHCl_3$ cast) 3034 (br), 3006 (m), 2979 (w), 2936 (w), 1790 (s), 1724 (s), 1514 (s), 1498 (m), 1455 (m), 1418 (m), 1395 (m), 1370 (m), 1343 (m), 1310 (m), 1218 (w), 1155 (s), 1061 (m), 755 (s), 698 (s) cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) δ 7.3 (m, 10H, aryl-H), 6.9 (s, br, 2H, CO_2H, OH), 6.0 (d, J = 6.8 Hz, 0.3H, NH minor isomer), 5.9 (d, J = 7.1 Hz, 0.7H, NH major isomer), 5.1 (m, 3H, CHOH, CH_2 benzyl ester), 4.4 (m, 0.3H, α-CH minor isomer), 4.1 (m, 0.7H, α-CH major isomer), 3.4 (m, 0.7H, β-CH major isomer), 3.2 (m, 0.3H, β-CH minor isomer), 1.5 (s, 3H, $(CH_3)_3C$ minor isomer), 1.4 (s, 6H, $(CH_3)_3C$ major isomer); ^{13}C NMR (75 MHz, $CDCl_3$) δ 128.8, 128.5, 128.1, 126.8, 126.2 (CH aryl), 72.9 (PhCOH), 67.6 ($PhCH_2O$), 54.8 (NCH), 27.8, 27.7(CH_3); MS (EI) calcd for $C_{16}H_{20}O_7N$, 338.1240; found 338.1035 (M-benzyl group) (0.13%), 91.0547 (100%), (FAB) 452 (MNa^+, 4%).

(3E,2R)-2-Benzyloxycarbonylamino-4-phenyl-3-butenoic Acid *tert*-Butyl Ester (19) and (3Z, 2R)-2-Benzyloxycarbonylamino-4-phenyl-3-butenoic Acid *tert*-Butyl Ester (20).[72]

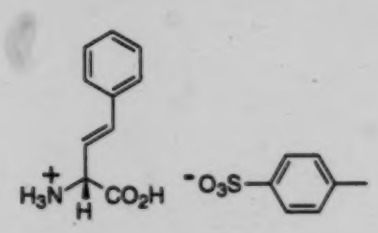


Compound **18** (1.7 g, 4.6 mmol) was dissolved in THF (30 mL), and triphenylphosphine (1.08 g, 4.1 mmol) was added to the solution. Diethyl azodicarboxylate (DEAD) was added dropwise until an orange colour persisted (about 1 mL). The solvents were then removed. The crude product was purified by flash chromatography (SiO_2, petroleum ether/Et_2O: 3/1, R_f 0.25) to give a clear oil that was 9:1 mixture of **19** and **20** $[\alpha]_D$ = -66.3 (c=0.3 in $CHCl_3$); IR (CH_2Cl_2 cast) 3335 (br, m), 2980 (m), 1725 (s), 1155 (s) cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) δ 7.3 (m, 10H, aryl-H), 6.8 (d, *J* = 15.9 Hz, 1H, arylCH=), 6.3 (d of d, *J* = 6.1, 15.9 Hz, 1H, CH=), 5.5 (s br, 1H, NH), 5.1 (s, 2H, CH_2 benzyl ester), 5.0 (t, *J* = 6.6 Hz, 1H, α-CH), 1.5 (s, 9H, $(CH_3)_3C$); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 169.5, 155.4, 136.2, 135.9, 132.3, 128.4, 128.3, 127.8, 126.4, 124.1, 82.4, 66.8, 56.2, 27.7; MS (posCI) $C_{22}H_{25}NO_4$, 368; found 369 (MH^+); Anal. Calcd for: $C_{22}H_{25}NO_4$: C, 71.91; H, 6.86; N, 3.81 Found: C, 72.0; H, 6.95; N, 3.8.

Further column chromatography (EtOAc:toluene, 1:99) allowed isolation of the (Z)-alkene **20** as a colourless oil: $[\alpha]_D$ = -162.5 (c=0.8 in $CHCl_3$); IR (CH_2Cl_2 cast) 3335 (br, m), 2980 (m), 1725 (s), 1155 (s) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.3 (m, 10H, aryl-H), 6.7 (d, *J* = 11.3 Hz, 1H, arylCH=), 5.5 (d of d, *J* = 9.8, 11.3 Hz, 1H, CH=), 5.5 (s, br, 1H, NH), 5.2 (t, *J* = 8.2 Hz, 1H, α-CH), 5.1 (s, 2H, CH_2 benzyl ester), 1.5 (s, 9H, $(CH_3)_3C$); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 169.9, 155.1, 136.2, 135.7, 134.1, 128.7,

128.5, 128.4, 128.3, 128.0, 127.6, 125.7, 82.5, 66.7, 53.0, 27.8; MS (FAB) $C_{22}H_{25}NO_4$, 367; found 368 (MH^+, 1.5%), 91 (100%).

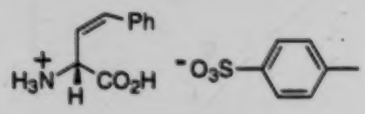
(3*E*, 2*R*)-2-Amino-4-phenyl-3-butenoic Acid *p*-Toluenesulfonate Salt (21).[28]



Trimethylsilyl iodide (100 μL, 0.70 mmol) was added to a solution of ester **19** (90 mg, 0.24 mmol) in $CHCl_3$ (5 mL) under Ar, and the mixture was stirred at room temperature for 1 h. Water (0.5 mL) was added and, the mixture was stirred for 10 minutes, and *p*-toluenesulfonic acid (48 mg, 0.25 mmol) was added. After stirring for a further 5 minutes, the reaction mixture was partitioned between water (10 mL) and $CHCl_3$ (10 mL), and the aqueous layer was washed with $CHCl_3$ (2x10 mL). The combined aqueous layers were removed to give the crude product. Recrystallisation from methanol/ether gave 44 mg (51%) of amino acid tosylate salt **21** as a yellow solid. Purification of the residue by reverse phase HPLC [(μBondapak); mobile phase, A, 0.1% TFA in water, B, 0.1%TFA in MeOH; 0 to 30% B over 20 min; ret. time 11.2 minutes] gave 14 mg (20%) of the trifluoroacetate salt as a pale yellow glass. Tosylate salt **21**; mp 209-210°C; $[\alpha]_D$ = -77 (c=0.031 in MeOH); IR (MeOH cast) 3415 (br, w), 3275-2800 (br m), 2925 (m), 1745 (m), 1230 (s), 1160 (s) cm-1; ^{1}H NMR (360 MHz, CD_3OD) δ 7.70 (d, *J* = 8.0, 2H, aryl-C<u>H</u>, tosylate), 7.45-7.48 (m, 2H, aryl-C<u>H</u>), 7.3 (m, 3H, aryl-C<u>H</u>), 7.21 (d, *J* =8.0 Hz, 2H, aryl-C<u>H</u>, tosylate), 6.91 (d, *J* = 15.9 Hz, 1H, CH=C<u>H</u>Ph), 6.27 (d of d, *J* = 15.9, 8.1 Hz, 1H, C<u>H</u>=CHPh), 4.70 (d of d, *J* = 8.1, 1.0 Hz, 1H, C<u>H</u>NH), 2.36 (s, 3H, C<u>H</u>$_3$); ^{13}C NMR (75.5 MHz, CD_3OD) δ 170.4, 141.7, 139.0, 136.6, 130.0, 129.8, 127.9,

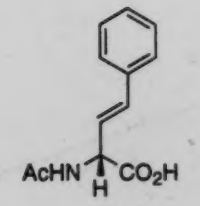
127.3, 126.9, 120.1, 55.9, 21.3; MS (electrospray) 178 (MH+, 55%), 161 (100%); HRMS calcd for $C_{10}H_{12}NO_2$, (MH^+) 178.0868; found 178.0870.

(3*Z*, 2*R*)-2-Amino-4-phenyl-3-butenoic Acid *p*-Toluenesulfonate Salt (22).[28]



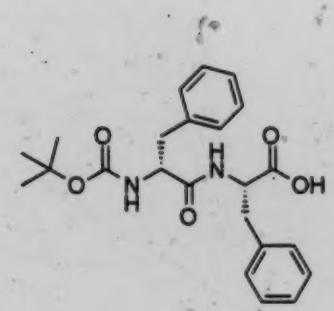
Trimethylsilyl iodide (100 µL, 0.70 mmol) was added to a solution of ester **20** (90 mg, 0.24 mmol) in $CHCl_3$ (5 mL) under Ar and the mixture was stirred at room temperature for 1 h. Water (0.5 mL) was added the mixture was stirred for 10 minutes, and *p*-toluene sulfonic acid (48 mg, 0.25 mmol) was added. After stirring for a further 5 minutes, the reaction mixture was partitioned between water (10 mL) and $CHCl_3$ (10 mL), and the aqueous layer was washed with $CHCl_3$ (2x10 mL). The aqueous layer was removed to give the product (80 mg, 93%) tosylate salt **22**: IR (µscope) 3350-2200 (br s), 1780 (m) and 1730 cm^{-1}; ^1H NMR (300 MHz, CD_3OD) δ 7.68 (d, J = 8.0 Hz, 2H, tolsylate C<u>H</u>), 7.3-7.5 (5H, m, aromatic), 7.2 (d, J = 8.0 Hz, 2H, tosylate <u>H</u>), 7.0 (d, J = 11.3 Hz, 1H, CH=C<u>H</u>Ph), 5.7 (t, J = 10.9 Hz, 1H, C<u>H</u>=CHPh), 5.0 (d, J = 10.5 Hz, 1H, C<u>H</u>NH), 2.3 (s, 3H, C<u>H</u>$_3$); ^{13}C NMR (75 MHz, CD_3OD) δ 170.5, 142.1, 139.2, 136.2, 130.0, 129.9, 129.8, 129.6, 127.0, 121.8, 52.0, 21.5; MS (electrospray) 876 ($3xM^+$ + 2 TsOH acid $+H^+$, 89%), 178 (MH^+, 82%); HRMS calcd for $C_{10}H_{12}NO_2$ (MH^+), 178.0868; found 178.0868.

(3*E*,2*R*)-*N*-Acetyl-2-amino-4-phenyl-3-butenoic Acid (23).[28]



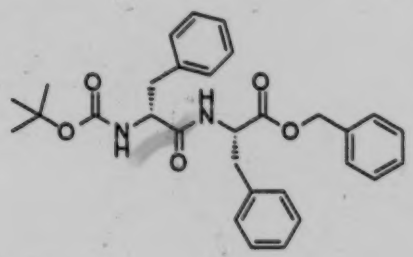
To a solution of amino acid **21** (56 mg, 0.16 mmol) in DMF (4 mL) was added acetyl chloride (20 μL, 0.28 mmol) followed by pyridine (35 μL, 0.43 mmol). The mixture was stirred at room temperature ander Ar for 16 hours, and was then partitioned between 1M HCl (10 mL) and EtOAc (10 mL). The aqueous layer was extracted with EtOAc (10 mL) and the combined organic layers were washed with brine (10 mL) and dried ($MgSO_4$). The solvent was removed. The residue was purifed by reverse phase HPLC (Resolve) mobile phase, A, 0.1% TFA in water, B, 0.1% TFA in CH_3CN; 0 to 30% B over 20 minutes; ret. time 14.5 minutes to give 11 mg (31%) of amide **23a** as a pale yellow oil: IR (MeOH cast) 3700-2300 (br, m), 3060 (m), 3030 (m), 1720 (s), 1660 (s), 1205 (s) cm^{-1}; ^1H NMR (300 MHz, CD_3OD) δ 7.23 - 7.41 (m, 5H, aryl-C<u>H</u>), 6.68 (d, J = 15.9 Hz, 1H, CH=C<u>H</u>Ph), 6.30 (d of d, J = 15.9, 6.8 Hz, 1H, C<u>H</u>=CHPh), 5.08 (d, J = 6.8 Hz, 1H, C<u>H</u>NH), 2.02 (s, 3H, C<u>H</u>$_3$); ^{13}C NMR (75.5 MHz, CD_3OD) δ 173.6, 173.0, 137.7, 134.4, 129.7, 129.1, 127.6, 124.5, 56.0, 22.4; MS (EI) calcd for $C_{12}H_{13}NO_3$, 219.0895; found 219.0894 (4%), 132 (100%).

_N_-Boc-D-phenylalanyl-L-phenylalanine (25).[64]



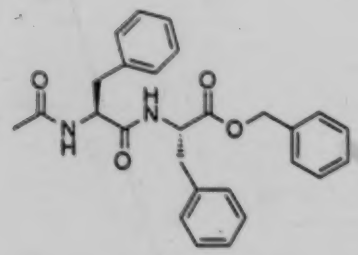
The literature procedure was modified.[64] *N*-Boc-L-phenylalanyl-L-phenylalanine benzyl ester (**115**) (1.00 g, 2.00 mmol) and 5% Pd/C (75 mg) was suspended in 1/1 EtOAc/MeOH (100 mL) and the mixture was stirred under H_2 (48 psi) for 1.5 hours. The mixture was filtered through celite and the solvent was removed to give **25** (0.78g, 95%): mp 62 °C; IR (KBr disk) 3450 (m), 3300 (br), 3080 (m), 3050 (m), 2975 (m), 2890 (m), 1721 (s), 1658 (s), 1528 (s), 1497 (s), 1392 (m), 1250 (m), 1188 (s), 699 (s) cm^{-1}; ^1H NMR (200 MHz, CD_3OD) δ 7.35-7.05 (m, 10H, aryl-H), 4.7 (br q, 1H, α–CH), 4.35 (br q, 1H, α–CH), 3.2 (d of d, J = 6.9, 1.0 Hz, 1H, β–CH_2), 3.0 (m, 2H, β–CH_2), 2.7 (m, 1H, β–CH_2), 1.35 (s, 9H, CH_3); MS (EI) calcd for $C_{20}H_{19}N_2O_3$, 339.1345; found 339.1342 (M-$(CH_3)_3CO_2$), 4.62%), 120.0813 (100%).

_N_-Boc-D-phenylalanyl-L-phenylalanine Benzyl Ester (28)[65].



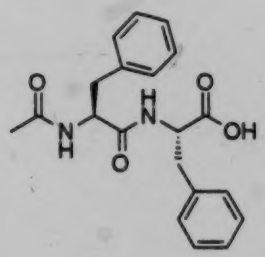
The general coupling procedure was modified.[40] _N_-Boc-D-phenylalanine (1.10 g, 4.15 mmol) and L-phenylalanine benzyl ester (1.71g, 4.00 mmol) were dried separately under vacuum for 16 hours. Dry DMF (total 25 mL) was added to both starting materials, the solutions were combined, cooled to 0°C and stirred under argon. Diphenylphosphoryl azide (1.10 mL, 5.05 mmol) was added followed by NEt_3 (1.50 mL, 11.0 mmol) in DMF (100 mL). The solution was stirred for 6 hours. The mixture was diluted with hexane (50 mL) and EtOAc (200 mL) and washed with 1N HCl (2x50 mL), H_2O (50 mL), saturated $NaHCO_3$ (2x50 mL), H_2O (50 mL) and saturated NaCl (2x50 mL). The organic layer was dried (Na_2SO_4) and the solvent was removed. The product was recrystallized from EtOAc/hexane to give **28** (1.05 g, 52%): mp 124-126 °C; IR (KBr disk) 3329 (s), 3100 (w), 3050 (w), 3000 (w), 2950 (w), 1735 (s), 1685 (s), 1658 (s), 1542 (m), 1528 (s), 1278 (m), 1166 (m), 701 (m) cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 7.40-7.10 (m, 13H, Ar-H), 6.9-6.8 (m, 2H, Ar-H), 6.40 (d, J = 7.0 Hz, 1H, NH), 5.10-5.12 (s, 2H, CH_2-benzyl ester), 4.95 (m, 2H, α–CH), 4.35 (d, J = 7.0 Hz, 1H, NH), 3.05 (m, 4H, β–CH_2), 1.4 (s, 9H, CH_3); MS (EI) calcd for $C_{30}H_{34}N_2O_5$, 502.2468; found 502.2475 (0.29%), 120.0814 (100%).

N-Acetyl-L-phenylalanyl-L-phenylalanine Benzyl Ester (30).



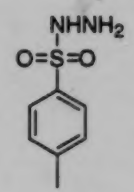
The general coupling procedure was modified.[40] *N*-Acetyl-L-phenylalanine (1.87 g, 9.00 mmol and L-phenylalanine benzyl ester (3.85 g, 9.00 mmol) were dried separately under vacuum for 16 hours. Dry DMF (total 25 mL) was added to both starting materials, the solutions were combined, cooled to 0°C and stirred under argon. Diphenylphosphoryl azide (2.30 mL, 10.6 mmol) was added followed by NEt_3 (2.90 mL, 21.3 mmol) in DMF (100 ml). The solution was stirred for 6 hours. The mixture was diluted with benzene (200 mL) and EtOAc (400 mL) and washed with 1N HCl (2x100 mL), H_2O (100 mL), saturated $NaHCO_3$ (2x100 mL), H_2O (100 mL) and saturated NaCl (2x100 mL). The organic layer was dried (Na_2SO_4) and the solvent was removed. The product was recrystallized from EtOAc/hexane to give **30** (2.40 g, 60%): mp 167-168 °C; IR (KBr disk) 3350 (br), 3289 (s), 3060 (w), 3010 (w), 1721 (m), 1640 (s), 1546 (m), 1500 (w), 1455 (m), 1445 (w), 1281 (m), 750 (m), 699 (s) cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 7.35-7.05 (m, 13 H, Ar-H), 6.9-6.8 (m, 2H, Ar-H), 6.15 (d, J = 7.0 Hz, 1H, NH), 6.00 (d, J = 7.1 Hz, 1H, NH), 5.05 (s, 2H, CH_2 benzyl ester), 4.75 (q, J = 6.8 Hz, 1H, α–CH), 4.55 (q, J = 7.0 Hz, 1H, α–CH), 2.95 (m, 4H, β–CH_2), 1.85 (s, 3H, $CH_3C(O)$-); MS (EI) calcd for $C_{27}H_{28}N_2O_4$, 444.2049; found 444.2044 (5.3%), 120.0814 (100%).

N-Acetyl-L-phenylalanyl-L-phenylalanine (32)



The literature procedure was modified.[64] *N*-Acetyl-L-phenylalanyl-L-phenylalanine benzyl ester (**30**) (2.40 g, 5.4 mmol) and 5% Pd/C (150 mg) were suspended in 80/20/0.1 MeCN/H_2O/TFA (100 mL), the mixture was hydrogenated while stirring under H_2 (48 psi) for 16 hours. The mixture was filtered through celite and the solvent was removed to give **117** (1.92g, 100%): mp 196-198 °C; IR (KBr disk) 3291 (s), 3100 (br), 3061 (m), 1729 (s), 1686 (s), 1639 (s), 1563 (s), 1253 (m), 700 (m) cm^{-1}; ^1H NMR (200 MHz, d_6-DMSO) δ 8.1 (t, *J* = 6.8 Hz, 2H, NH), 7.3-7.1 (m, 10H, aryl-H), 4.6-4.3 (m, 2H, α-CH), 3.0 (m, 4H, β-CH_2), 1.75 (s, 3H, $CH_3C(O)$; MS (EI) calcd for $C_{20}H_{22}N_2O_4$, 354.1580; found 354.1580 (4.3%), 120.0807 (100%).

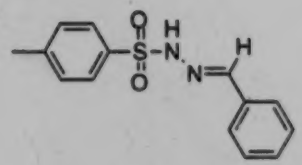
Tosylhydrazine (34)[66].



The literature procedure was modified.[66] Tosyl chloride (100 g, 0.52 mol) was dissolved in THF (300 mL) and added to a 3 neck flask fitted with a thermometer and dropping funnel charged with 33.5 mL N_2H_4 (anhydrous) and 33.5 mL of H_2O. The flask was

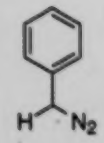
cooled in an ice bath and the hydrazine was added so that the temperature did not exceed 15°C, the mixture was then stirred for a further 30 minutes. The mixture was diluted with 1.5 L of $CHCl_3$ and washed with saturated NaCl (2x200 mL). The organic layer was dried with Na_2SO_4 and the solvent was removed to give the product **34** (70 g, 72%): mp 100-102°C; IR ($CHCl_3$ cast) 3258 (m), 1596 (w), 1419 (w), 1306 (m), 1155 (s), 814 (m) cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 7.9 (d, *J* =8.0, 2H, aryl-H), 7.4 (d, *J* = 8.0, 2H, aryl-H), 6.0 (s, br, 1H, NH), 3.5 (s, br, 2H, NH_2) 2.4 (s, 3H, CH_3); MS (posCI) $C_7H_{10}N_2O_2S$, 186; found 187 (MH^+).

Benzaldehyde Tosyl Hydrazone (35)[66].



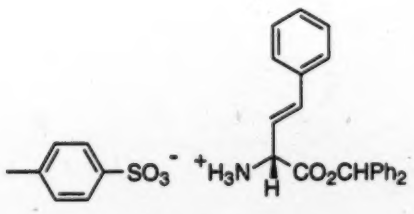
The literature procedure was modified.[66] Tosylhydrazine (**34**) (58.4 g, 0.31 mol) was added to a 500 mL flask and dissolved in MeOH (300 mL). Freshly distilled benzaldehyde (30 mL, 0.30 mol) was added rapidly and the mixture was swirled until dissolved. It was allowed to stand for 30 minutes and was then cooled to 0°C to cause the product to crystallize. The crystals were collected by vacuum filtration and dried under high vacuum to give the product **35** (65 g, 79%): mp 129-131°C; IR ($CHCl_3$ cast) 3224 (m), 1437 (w), 1365 (m), 1324 (m), 1306 (m), 1291 (m), 1161 (s), 1042 (m), 956 (m), 666 (m) cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 8.4 (s br, 1H, NH), 7.9 (d, *J* = 7.6, 2H, α-CH ortho to SO_2), 7.8 (s, 1H, CH=), 7.6 (m, 2H, aryl-H), 7.3 (m, 5H, aryl-H), 2.4 (s, 3H, CH_3); MS (posCI) $C_{14}H_{14}N_2SO_2$, 274; found 275 (MH^+).

Phenyldiazomethane (36)[66].



Sodium methoxide (100 mL, 1M) was prepared by dissolving Na (2.3 g, 0.10 mol) in 100 mL of MeOH. Benzaldehyde tosylhydrazone (**35**) (13.8 g, 0.050 mol) was placed in a 250 mL round bottom flask and a solution of NaOMe in MeOH (54 mL of a 1M solution, 0.054 mol) was added. The flask was swirled until **35** had dissolved. The solvent was removed and the resulting solid was dried under high vacuum for 16 hours. The solid was then broken up with a spatula and the flask was immersed in an oil bath and fitted with a still head, a vacuum takeoff adapter and a 50 mL receiver flask. The receiver flask was cooled in a dry ice/acetone bath. The system was evacuated and the flask was heated to 90°C. The temperature was then slowly increased to 160°C over 5 hours. The product **36** was collected in the receiving flask as a red liquid and was stored at -70°C as a solid until needed.

(3*E*, 2*R*) 2-Amino-4-phenyl-3-butenoic Acid Diphenylmethyl Ester *p*-Toluene sulfonate Salt (37).[28]



To a solution of amino acid salt (**21**) (22 mg, 63 μmol) in DMF (2mL) at 50°C was added a solution of diphenyldiazomethane (18.6 mg, 96 μmol) in DMF (1mL). The mixture was stirred at 50°C for 15 minutes and the solvent was removed. Recrystallisation from acetonitrile gave 17.7 mg (55%) of the ester **37** as a colourless solid; mp 170-173°C; $[\alpha]_D$ = -7±8 (c=0.06 in MeOH); IR 3430 (br, m), 3400 (br m), 3350-2400 (br, s), 3060 (s), 2925 (s), 1745 (s) cm^{-1}; ^1H NMR (360 MHz, CD_3OD) δ 7.70 (d, *J* = 8.2 Hz, 2H, aryl-C<u>H</u>, tosylate), 7.19-7.41 (m, 17H, m, aryl-C<u>H</u>), 6.99 (s, 1H, C<u>H</u>Ph_2), 6.94 (d, *J* = 15.9 Hz, 1H, CH=C<u>H</u>Ph), 6.22 (d of d, *J* = 15.9, 8.3, 1H, C<u>H</u>=CHPh), 4.94 (d of d, *J* = 8.3, 0.8, 1H, C<u>H</u>NH), 2.35 (s, 3H, $C\underline{H}_3$); MS (FAB) 366 (MNa^+, 8%), 167 (100%); HRMS calcd for $C_{12}H_{13}NO_3$, 219.0895; found 219.0894.

Preparation of Styrylglycine Tripeptides 38-41.[28]

Method A. To a solution of *N*-Boc-D-Phenylalanyl-L-Phenylalanine (**25**) (12.2 mg, 30.0 μmol) in DMF (1 mL), under Ar at 0°C was added benzotriazol-1-yloxy-tris(dimethylamino)phosphonium hexafluorophosphate (BOP reagent) (14.4 mg, 33.0 μmol) followed by triethylamine (4.2 μL, 30 μmol). The mixture was stirred at 0°C for 50 minutes then a solution of amino ester (**37**) (14.5 mg, 28 μmol) in DMF (1 mL) was added followed by triethylamine (3 μL, 20 μmol). The mixture was allowed to warm to room temperature and stirred for 15 h before being partitioned between CH_2Cl_2 (10 mL) and

NH_4Cl (aq) (10 mL). The aqueous layer was washed with CH_2Cl_2 (10 mL) and the combined organic extracts were dried ($MgSO_4$) and the solvent was removed. Purification by column chromatography (EtOAc:hexane, 1:4) gave 13.2 mg of protected tripeptide which was stirred in trifluoroacetic acid (3 mL) at 0°C for 80 minutes. Solvent was removed and the residue was purified by reverse phase HPLC to give 4.1 mg (25%) of tripeptide (**38**), 1.9 mg (12%) of tripeptide (**39**), 2.5 mg (15%) of tripeptide **40** and 1.9 mg (12%) of tripeptide **41**.

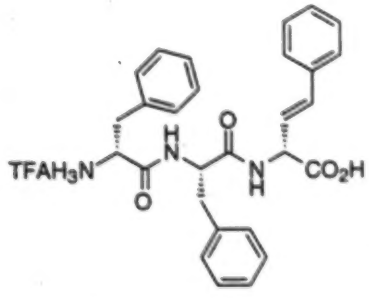
Method B. To a solution of *N*-Boc-D-Phenylalanyl-L-Phenylalanine (14 mg, 34 μmol) in DMF (1mL) and CH_2Cl_2 (1mL), under Ar at 0°C, was added 3-ethyl-1-(3-dimethylaminopropyl)carbodiimide hydrochloride (6.5 mg, 34 μmol) followed by 1-hydroxy-7-azabenzotriazole (4.6 mg, 34 μmol). The mixture was stirred at 0°C for 20 min, and then a solution of amino ester **37** (7.0 mg, 14 μmol) in DMF (100 μL) was added followed by collidine (1.6 μL, 12 μmol). The mixture was allowed to warm to room temperature and stirred for 15 hours before being partitioned between CH_2Cl_2 (10 mL) and NH_4Cl (aq) (10 ml). The aqueous layer was washed with CH_2Cl_2 (10 ml), the combined organic extracts were dried ($MgSO_4$) and the solvent was removed. Purification by column chromatography (EtOAc:hexane, 1:4) gave 7.6 mg (76%) of *N-tert*-butyloxycarbonyl-D-phenylalanyl-L-phenylalanyl-(3*E*)-2-amino-4-phenyl-3-butenoic acid diphenylmethyl ester as a colourless oil; R_f = 0.16 (EtOAc:hexane, 3:7); IR ($CHCl_3$ cast) 3285 (br m), 3060 (w), 3030 (w), 2975 (w), 2930 (w), 17454 (m), 1695 (m), 1645 (s), 700 (s) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) (4:3 ratio of diastereomers) δ 7.40-7.12 (m, 23H, aryl-C<u>H</u>), 6.98 (m, 2H, aryl-C<u>H</u>), 6.90 (br, 1H, N<u>H</u>, minor), 6.87 (s, 1H, C<u>H</u>Ph_2, major), 6.86 (s, 1H, C<u>H</u>Ph_2), 6.77 (br, 1H, N<u>H</u>), 6.52 (d of d, *J* = 15.9, 1.3 Hz, 1H, CH=C<u>H</u>Ph), 6.46 (d, *J* = 15.0 Hz, 1H, CH=C<u>H</u>Ph, minor), 6.31 (d, *J* = 7.2 Hz, 1H, N<u>H</u> major), 6.22 (d, *J* = 6.9 Hz, 1H, N<u>H</u> minor), 6.08 (d of d, *J* = 15.9, 6.7 Hz, 1H, C<u>H</u>=CHPh, major), 5.99 (d of d, *J* = 15.9, 7.1 Hz, 1H, C<u>H</u>=CHPh, minor), 5.32 (t, *J* = 7.1 Hz, C<u>H</u>CH=CH,

minor), 5.26 (d of t, J = 7.0,1.3 Hz, 1H, C<u>H</u>CH=CH, major), 4.91 (br, 1H, N<u>H</u>), 4.76-4.67 (m, 1H, C<u>H</u>NH), 4.20 (q, J = 7.2 Hz, 1H, C<u>H</u>NH, major), 4.12 (q, J = 7.1 Hz, 1H, C<u>H</u>NH, minor), 3.13-2.88 (m, 2H, C<u>H</u>$_2$Ph), 2.80-2.75 (m, 1H, C<u>H</u>NH), 1.29 (s, 9H, C(C<u>H</u>$_3$)$_3$, major), 1.26 (s, 9H, C(C<u>H</u>$_3$); MS (electrospray) 760 (MNa^+, 71%), 738 (MH^+), 167 (100%).

A solution of protected tripeptide (7.6 mg, 10 μmol) in trifluoroacetic acid (2 mL) at 0°C was stirred for 75 minutes and the solvent was removed. Purification by reverse phase HPLC gave 2.6 mg (43%) of tripeptide as its TFA salt along with 3.4 mg (56%) of tripeptide as its TFA salt.

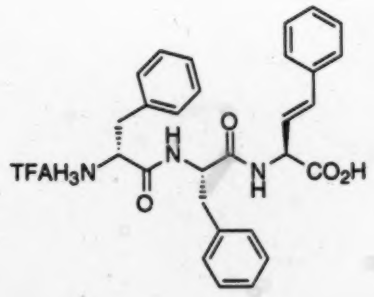
Method C. To a solution of *N*-Boc-D-phenylalanyl-L-phenylalanine (4.8 mg, 12 μmol) in DMF (1 mL), under Ar at 0°C, was added 3-ethyl-1-(3-dimethylamino propyl)carbodiimide hydrochloride (2.3 mg, 12 μmol) followed by 1-hydroxy-7-azabenzotriazole (1.6 mg, 12 μmol). The mixture was stirred at 0°C for 20 minutes, and then amino acid **21** (4.0 mg, 11 μmol) was added followed by collidine (3 μL, 20 μmol). The mixture was allowed to warm to room temperature and stirred for 5 hours before being partitioned between EtOAc (10 mL) and 1M HCl (10 mL). The aqueous layer was washed with EtOAc (10 mL), the combined organic extracts were dried ($MgSO_4$) and the solvent was removed. The residue was purified by reverse phase HPLC to give 1.3 mg (19%) of tripeptide **38** as the only isolated product. Analysis of the crude product by HPLC indicated a diastereomeric excess (d.e.) of >86%.

D-Phenylalanyl-L-phenylalanyl-(3*E*, 2*R*)-2-amino-4-phenyl-3-butenoic Acid Trifluoroacetate Salt (38).[28]



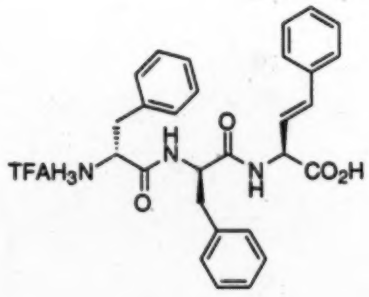
(Waters, Resolve; mobile phase, A, 0.1% TFA in water, B, 0.1% TFA in CH_3CN; 25 to 50% B over 29 minutes; ret. time 25.0 minutes; IR (MeOH cast) 3600-2800 (br m), 2930 (m), 1675 (s), 1205 (s), 1145 (s) cm^{-1}; ^1H NMR (300 MHz, CD_3OD) δ 7.08-7.37 (15H, m, aromatic), 6.50 (d of d, *J* = 15.9, 0.9 Hz, 1H, CH=C<u>H</u>Ph), 6.16 (d of d, *J* = 15.9, 6.8 Hz, 1H, C<u>H</u>=CHPh), 5.02 (d of d, *J* = 6.8, 1.3 Hz, 1H, C<u>H</u>CH=CH), 4.78 (d of d, *J* = 8.4, 6.8 Hz, 1H, C<u>H</u>NH), 4.07 (d of d, *J* = 8.4, 6.8 Hz, 1H, C<u>H</u>NH), 3.04 (d of d, *J* = 13.6, 6.8 Hz, 1H, C<u>H</u>$_2$Ph), 2.97 (d of d, *J* = 14.1, 6.3 Hz, 1H, C<u>H</u>$_2$Ph), 2.76-2.83 (m, 2H); ^{13}C NMR (75.5 MHz, CD_3OD) δ 172.6, 137.9, 135.4, 134.5, 130.5, 130.1, 129.7, 129.6, 129.2, 128.9, 128.1, 127.7, 124.2, 55.8, 55.6, 39.4, 38.5; MS (FAB) 494 (MNa^+, 4.5%), 472 (MH^+, 1%), 87 (100%); HRMS (electrospray) calcd for $C_{28}H_{30}N_3O_4$ (MH+), 472.2236; found 472.2228.

D-Phenylalanyl-L-phenylalanyl- (3*E*, 2*S* 2-amino-4-phenyl-3-butenoic Acid Trifluoroacetate Salt (39).[28]



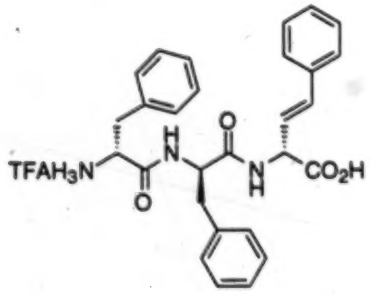
(Waters, Resolve; mobile phase, A, 0.1% TFA in water, B, 0.1% TFA in CH_3CN; 25 to 50% B over 29 minutes; ret. time 29.0 minutes; IR (MeOH cast) 3600-2800 (br m), 2925 (m), 1675 (s), 1205 (s), 1140 (s) cm^{-1}; ^1H NMR (300 MHz, CD_3OD) δ 7.18-7.42 (15H, m, aromatic), 6.98-7.01 (m, 2H, aryl-C<u>H</u>), 6.72 (d, *J* = 15.9 Hz, 1H, CH=C<u>H</u>Ph), 6.32 (d of d, *J* = 15.9, 6.4 Hz, 1H, C<u>H</u>=CHPh), 5.10 (d of d, *J* = 6.4, 1.1 Hz, 1H, C<u>H</u>CH=CH), 4.78 (d of d, *J* = 10.0, 4.9 Hz, 1H, C<u>H</u>NH), 4.05 (d of d, *J* = 7.8, 5.8 Hz, 1H, C<u>H</u>NH), 3.20 (d of d, *J* = 14.0, 4.9 Hz, 1H, C<u>H</u>$_2$Ph), 2.92 (d of d, *J* = 14.2, 5.8 Hz, 1H, C<u>H</u>$_2$Ph), 2.84 (d of d, *J* = 14.0, 10.0, 1H), 2.73 (d of d, *J* = 14.2, 7.8, 1H); ^{13}C NMR (100.6 MHz, CD_3OD) δ 169.5, 138.2, 137.6, 135.2, 134.4, 130.4, 130.3, 130.1, 129.7, 129.6, 129.2, 128.9, 128.1, 127.6, 124.2, 56.0, 55.5, 39.1, 38.4; MS (FAB) 494 (MNa^+, 1.5%), 472 (MH^+), 120 (100%); HRMS (electrospray) calcd for $C_{28}H_{30}N_3O_4$ (MH+), 472.2236; found 472.2238.

D-Phenylalanyl-D-phenylalanyl-(3*E*, 2*S*)-2-amino-4-phenyl-3-butenoic Acid Trifluoroacetate Salt (40).[28]



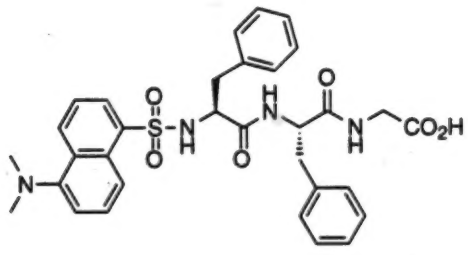
(Waters, Resolve; mobile phase, A, 0.1% TFA in water, B, 0.1% TFA in CH_3CN; 25 to 50% B over 29 minutes; ret. time 19.4 minutes; IR (MeOH cast) 3700-2300 (br m), 1670 (s), 1205 (s), 1145 (s) cm^{-1}; ^1H NMR (360 MHz, CD_3OD) δ 7.14-7.36 (15H, m, aromatic), 6.42 (d, *J* = 15.9 Hz, 1H, CH=C<u>H</u>Ph), 6.12 (d of d, *J* = 15.9, 6.9 Hz, 1H, C<u>H</u>=CHPh), 5.03 (d, *J* = 6.9 Hz, 1H, C<u>H</u>CH=CH), 4.78-4.74 (m, 1H, C<u>H</u>NH), 4.08 (d of d, *J* = 8.1, 5.6 Hz, 1H, C<u>H</u>NH), 3.24 (d of d, *J* = 14.5, 5.6 Hz, 1H, C<u>H</u>$_2$Ph), 3.12-2.92 (m, 3H); ^{13}C NMR (75.5 MHz, CD_3OD) δ 133.8, 130.2, 130.0, 129.4, 129.3, 128.7, 128.4, 127.7, 127.4, 124.2, 56.1, 55.2, 39.2, 38.7; MS (electrospray) 494 (MNa^+, 100%), 472 (MH^+); HRMS (electrospray) calcd for $C_{28}H_{30}N_3O_4$ (MH+), 472.2236; found 472.2235.

D-Phenylalanyl-D-phenylalanyl-(3*E*- 2*R*)-2-amino-4-phenyl-3-butenoic Acid Trifluoroacetate Salt (41).[28]



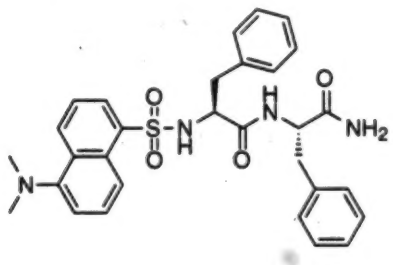
(Waters, Resolve; mobile phase, A, 0.1% TFA in water, B, 0.1% TFA in CH_3CN; 25 to 50% B over 29 minutes; ret. time 16.7 minutes; IR (MeOH cast) 3700-2650 (br m), 2550 (br m), 1670 (s), 1205 (s), 1145 (s) cm^{-1}; ^1H NMR (360 MHz, CD_3OD) δ 7.40-7.43 (m, 2H, aryl-C<u>H</u>), 7.17-7.33 (13H, m, aromatic), 6.73 (d, *J* = 16.0 Hz, 1H, CH=C<u>H</u>Ph), 6.34 (d of d, *J* = 16.0, 6.5 Hz, 1H, C<u>H</u>=CHPh), 5.11 (d of d, *J* = 6.5, 1.5 Hz, 1H, C<u>H</u>CH=CH), 4.80 (d of d, *J* = 9.1, 5.6 Hz, 1H, C<u>H</u>NH), 4.05 (d of d, *J* = 8.6, 5.1 Hz, 1H, C<u>H</u>NH), 2.98 (d of d, *J* = 14.4, 8.6 Hz, 2H, C<u>H</u>$_2$Ph), 3.28-3.20 (m, 2H, C<u>H</u>$_2$Ph); ^{13}C NMR (HMQC, 125.7 MHz (1H, 500 MHz), CD_3OD) δ 134.1, 130.2, 130.0, 129.4, 129.3, 128.7, 127.7, 127.4, 124.2, 55.9, 55.6, 55.1, 38.3; MS (electrospray) 494 (MNa^+, 4.5%), 472 (MH^+); HRMS (electrospray) calcd for $C_{28}H_{30}N_3O_4$ (MH+), 472.2236; found 472.2239.

***N*-Dansyl-L-phenylalanyl-L-phenylalanyl-glycine (42).**[11a]



The literature procedure was modified.[45] L-phenylalanyl-L-phenylalanyl-glycine trifluoroacetate salt (**45**) (50 mg, 0.10 mmol) was dissolved in 100 mL of carbonate buffer (0.1 M $NaHCO_3/Na_2CO_3$, pH 8.5) to a concentration of 1 μmol/mL. Dansyl chloride (100 mg, 0.37 mmol) in 100 mL of acetone was added to the carbonate buffer solution, and the mixture was stirred until the yellow colour faded (about 4 hours). The mixture was extracted with EtOAc (2x50 mL), the aqueous layer was acidified with 6 M HCl to pH 3 and was extracted with EtOAc (3x50 mL). The solvent was removed to give the crude product. This was purified by reverse phase HPLC (μBondapak, RP C-18, monitored at 254 nm); solvent A: 0.1% TFA in water, solvent B 80/20/0.1 MeCN/H_20/TFA; starting conditions 70/30 A/B changing to 40/60 A/B over 20 minutes, holding for 5 minutes, reverting to 70/30 A/B in 5 minutes and holding for 5 minutes. The product **42** (44.9 mg, 90%) had a retention time of 11.5 minutes: IR (CH_2Cl_2 cast) 3240 (br), 2925 (s), 1716 (s), 1662 (s), 1558 (br), 1455 (br), 1312 (m), 1231 (s), 1161 (s), 754 (s), 699 (s) cm^{-1}; ^1H NMR (200 MHz, CD_3OD) δ 8.5 (d, J = 8.8 Hz, 1H, aryl-H), 8.4 (d of t, J = 8.5, 1.0Hz, 1H, aryl-H), 8.1 (d of d, J = 6.8, 1.3 Hz, 1H, aryl-CH) 8.0 (m, 1H, aryl-CH), 7.5 (m, 2H, aryl-H), 7.2 (m, 8H, aryl-H), 6.8 (m, 2H, aryl-H), 4.0-3.8 (m, 4H, α-CH), 3.3-2.8 (m, 4H, β-CH_2), 2.9 (s, 6H, $(CH_3)_2N$), MS (EI) calcd for $C_{32}H_{34}N_4O_6S$ 602.2199 found 602.2220. (2.9%), 91.0549 (100%).

***N*-Dansyl-L-phenylalanyl-L-phenylalanine Amide (43).[11a]**

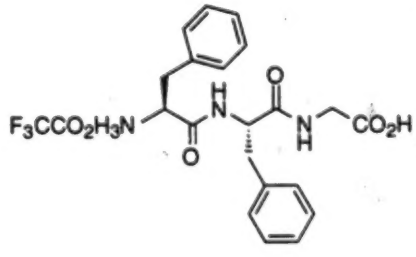


Method 1[11a]: L-Phenylalanyl-L-phenylalanine amide trifluoroacetate salt (20 mg, 0.047 mmol) was dissolved in carbonate buffer (0.1 M $NaHCO_3/Na_2CO_3$, pH 8.5, 40 mL) to a concentration of approximately 1 μmol/mL. Dansyl chloride (40 mg, 0.15 mmol) in 40 mL of acetone was added to the carbonate buffer solution and the mixture was stirred until the yellow colour faded (about 4 hours). The product was purified by reverse phase HPLC (μBondapak, RP C-18, monitored at 254 nm); solvent A: 0.1% TFA in water, solvent B 80/20/0.1 MeCN/$H_2$0/TFA; starting conditions 70/30 A/B changing to 40/60 A/B over 20 minutes, holding for 5 minutes, reverting to 70/30 A/B in 5 minutes and holding for 5 minutes. The product **43** (2 mg, 7.8%) had a retention time of 13.2 minutes:

Method 2[40]: Dansyl-L-phenylalanine (200 mg, 0.50 mmol) and L-phenylalanine amide (82 mg, 0.50 mmol) were dried separately under vacuum for 16 hours. Dry DMF (total 10 mL) was added to both starting materials. The solutions were combined and cooled to 0°C and stirred under argon. Diphenylphosphoryl azide (130 μL, 0.60 mmol) was added followed by NEt_3 (160 μL, 1.2 mmol) in DMF (0.5 mL). The solution was stirred for 6 hours. The mixture was diluted with hexane (50 mL) and EtOAc (100 mL), washed with 1N HCl (2x25 mL), H_2O (25 mL), saturated $NaHCO_3$ (2x25 mL), H_2O (25 mL) and saturated NaCl (2x25 mL). The organic layer was dried (Na_2SO_4) and the solvent was removed. The product was purified by flash chromatography (SiO_2; Et_2O, R_f 0.40) to give **43** (140 mg, 50%): IR ($CHCl_3$ cast) 3400-3100 (br, m), 2970 (m), 1639 (s), 1492

(m), 1454 (m), 1397 (m), 1382 (m), 1310 (m), 1198 (m), 1182 (m), 1145 (s), 1090 (m), 1078 (m) 1061 (m), 936 (m), 791 (s), 755 (s) cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 8.5 (d, *J* = 7.7 Hz, 1H, aryl-H), 8.3 (d, *J* = 7.3 Hz, 1H, aryl-H), 8.2 (d of d, *J* = 7.3, 1.3 Hz, 1H, aryl-H), 7.6-7.0 (m, 14H, aryl-H, NH), 5.9 (d, *J* = 7.0, 1H, NH), 4.4 (m, 2H, α-CH), 3.9 (s, 2H, NH_2), 2.9 (m, 10H, β-CH_2, $(CH_3)_2N$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.8, 170.7(2xCO amide), 151.9, 136.7, 134.3, 131.6, 131.2, 130.3, 129.7, 129.2, 129.0, 128.8, 128.6, 128.4, 128.2, 127.0, 126.9, 122.9, 117.6, 115.2 (aromatic C), 58.9 (NCH_3), 53.7, 45.3, 37.6, 37.3 (α, β CH); MS (posFAB) $C_{30}H_{32}O_4N_4S$, 544; found 545 (MH^+, 9.1%); Anal. Calcd for $C_{30}H_{32}N_4O_4S$: C, 66.15; H, 5.92; N, 10.29; O, 11.75; S, 5.89. Found C, 65.77; H, 5.73; N, 10.1.

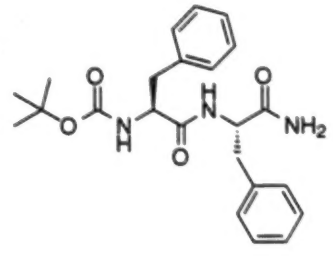
L-Phenylalanyl-L-phenylalanyl-glycine Trifluoroacetate Salt (45)[66].



The general deprotection procedure was modified.[43] *N*-Boc-L-phenylalanyl-L-phenylalanyl-glycine (100 mg, 0.20 mmol) was stirred in trifluoroacetic acid (5 mL) for 2 hours. The solvent was removed to give **45**, as a colourless oil (100 mg, 98%): mp 190-194 °C; IR (μscope) 3344 (br, m), 3258 (m), 3029 (m), 2924 (m), 1691 (m), 1631 (s), 1525 (s), 1497 (m), 1200 (m), 1156 (m), 1132 (s) cm^{-1}; ^1H NMR (360 MHz, D_2O) δ 6.85 (m, 10H, aryl-H), 4.75 (q, *J* = 8.5, 6.0 Hz, 1H, α-CH), 4.1 (q, *J* = 8.5, 5.5 Hz, 1H, α-CH), 3.9 (s, 2H, α-CH), 3.3-3.0 (ABX, *J* = 14.0, 8.5, 6.0, 2H, β-CH_2), 3.3-3.0 (ABX, *J* = 14.5, 8.5, 5.5 Hz, 2H, β-CH_2); ^{13}C NMR (75 MHz, CD_3OD) δ 173.2 ,

172.5, 169.6 (3x<u>C</u>O amide), 138.1, 135.4, 130.6, 130.3, 130.0, 129.5, 128.8, 127.9 (aromatic <u>C</u>), 56.2, 55.4 (2x<u>C</u>H_2), 41.8, 38.9, 38.5 (3xα-<u>C</u>H); MS (posFAB) $C_{20}H_{23}N_3O_4$, 368; found 369.8 (MH+, 31%), 91.0 (100%).

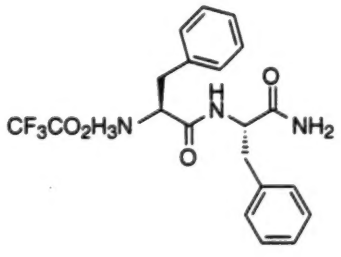
_N_-Boc-L-Phenylalanyl-L-phenylalanyl amide (46).



The general coupling procedure was modified.[40] _N_-Boc-L-phenylalanine (130 mg, 0.49 mmol) and phenylalanine amide (80 mg, 0.49 mmol) were dried separately under vacuum for 16 hours. Dry DMF (total 25 mL) was added to both starting materials, and the solutions were combined, cooled to 0°C and stirred under argon. Diphenylphosphoryl azide (0.12 mL, 0.50 mmol) was added followed by NEt_3 (0.16 mL, 1.1 mmol) in DMF (0.5 mL). The solution was stirred for 6 hours. The mixture was diluted with hexane (50 mL) and EtOAc (100 mL), washed with 1N HCl (2x25 mL), H_2O (25 mL), saturated $NaHCO_3$ (2x25 mL), H_2O (25 mL) and saturated NaCl (2x25 mL). The organic layer was dried (Na_2SO_4) and the solvent was concentrated to 5 mL. The crystalline product was filtered, washed with a small amount of EtOAc and dried under high vacuum to give **46** (0.070 g, 35%): mp 191-192°C; IR ($CHCl_3$ cast) 3355 (br), 3278 (br), 2169 (m), 1705 (s), 1687 (s), 1647 (s), 1523 (m), 1505 (m), 1489 (m), 1180 (m), 965 (m) cm^{-1}; ^1H NMR (200 MHz, d_7-DMF) δ 7.45-7.0 (m, 10H, aryl-<u>H</u>), 6.4 (br s, 1H, N<u>H</u>), 5.4 (br s, 1H, N<u>H</u>), 4.8 (d of q, J = 6.8, 1.5 Hz, 1H, α-C<u>H</u>), 4.2 (d of q, J = 6.9, 1.3 Hz, 1H, α-C<u>H</u>),

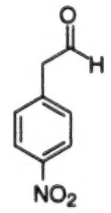
3.0 (m, 4H, β-C<u>H</u>$_2$), 1.3 (s, 9H, (C<u>H</u>$_3$)$_3$C); MS (EI) calcd for $C_{23}H_{29}N_3O_4$, 411.2158; found 411.2164 (1.05%), 57.0725 (100%).

L-Phenylalanyl-L-phenylalanine amide Trifluoroacetate Salt (47).



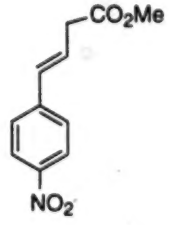
The general deprotection procedure was modified.[43] *N*-Boc-L-phenylalanyl-L-phenylalanyl amide (**46**) (69 mg, 18 mmol) was dissolved in trifluoroacetic acid (5 mL) and stirred for 4 hours. The solvent was removed to give the product **47** (51 mg, 96%): mp 191-192 °C; IR (KBr disk) 3399 (br), 3307 (s), 3210 (m), 3033 (w), 2933 (w), 1685 (s), 1667 (s), 1644 (s), 1560 (m), 1205 (s), 1190 (s), 1160 (s), 1139 (m), 722 (m), 700 (m) cm^{-1}; ^{1}H NMR (300 MHz, D_2O) 7.4-7.2 (m, 10H, aryl-<u>H</u>), 4.7 (q, *J* = 6.8 Hz, 1H, α-C<u>H</u>), 4.1 (q, *J* = 7.1 Hz, 1H, α-C<u>H</u>), 3.4-2.9 (m, 4H, β-C<u>H</u>$_2$); MS (EI) calcd for $C_{18}H_{21}N_3O_2$, 311.1634; found 311.1633. (0.78%), 120.0846 (100%).

(4-Nitrophenyl)acetaldehyde (49).[35]



4-Nitrostyrene (Pfaltz & Bauer, 6.80 g, 45 mmol) was dissolved in CH_2Cl_2 (90 mL). This solution was added to $Pb(OAc)_4$ (20.4 g, 46 mmol) in trifluoroacetic acid (45 mL), and stirred for 2 hours. The mixture was then washed with H_2O (50 mL), and saturated $NaHCO_3$ (50 mL). The solvent was dried with $MgSO_4$ and removed to yield the product **49** as a yellow oil (5.32 g, 79%): IR ($CHCl_3$ cast) 3180 (w), 3000 (w), 2870 (w), 1710 (s), 1608 (s), 1598 (s), 1519 (s), 1388 (m), 1342 (s), 1316 (s), 1177 (m), 1106 (s), 858 (s), 719 (s) cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 9.8 (t, J = 1.8 Hz, 1H, C<u>H</u>O), 8.2 (d, J = 8.7 Hz, 2H, aryl C<u>H</u> ortho to NO_2), 7.4 (d, J = 8.7 Hz, 2H, aryl C<u>H</u> meta to NO_2), 3.9 (d, J = 1.8 Hz, 2H, C<u>H</u>$_2$); MS (EI) calcd for $C_8H_7NO_3$, 165.0425; found 165.0426 (77.8%), 91.0548 (100%).

Methyl (4-Nitrophenyl)-3-butenoate (50)[54].

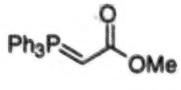


Method 1:[54] Ketone **91** (600 mg, 3.24 mmol), MeOH (2.34 µL, 9.6 mmol) and $BF_3 \cdot Et_20$ (2.45 mL) were dissolved in benzene (15 mL). This solution was added to a

suspension of $Pb(OAc)_4$ (1.66 g, 3.78 mmol, in 25 mL benzene) and the mixture was stirred under Ar for 18 hours. The solvent was removed, the residue was taken up in EtOAc (70 mL) and washed with H_20 (50 mL), saturated $NaHCO_3$ (50 mL), H_2O (1x50 mL) and saturated NaCl (50 mL). The solution was dried, the solvent was removed and the crude product was purified twice by chromatography (Florisil, 1% EtOAc in hexane to 100 % EtOAc, R_f 0.25 in 20% EtOAc in hexane) to give the product **50** as a yellow solid (238 mg, 33%).

Method 2:[36] (4-Nitrophenyl)acetaldehyde (**49**, 500 mg, 3.0 mmol) was dissolved in THF (30 mL), Ph_3PCHCO_2Me (**51**) (1.0 g, 3.0 mmol) was added and the solution was stirred for 16 hours. The solvent was removed and the residue was purified by column chromatography (Florisil, 10% EtOAc in hexane to 100% EtOAc, R_f 0.8) to yield the product **50** (668 mg, 80%): IR ($CHCl_3$ cast) 2940 (w), 1737 (s), 1596 (m), 1516 (s), 1436 (m), 1344 (s), 1299 (w), 1272 (w), 1256 (m), 1202 (m), 1167 (m), 1110 (m), 971 (m), 860 (w), 745 (m) cm^{-1}; ^1H NMR (360 MHz, d_6-benzene) δ 7.8 (d, J = 8.8 Hz, 2H, aryl-C<u>H</u> ortho to NO_2), 6.7 (d, J = 8.8 Hz, 2H, aryl-C<u>H</u> meta to NO_2), 6.1 (d of t, J = 16.1, 7.0 Hz, 1H, C<u>H</u>=CH_2), 5.9 (d, J = 16.1 Hz, 1H, C<u>H</u>=CH), 3.3 (s, 3H, $C\underline{H}_3$), 2.8 (d of d, J = 7.0, 1.2 Hz, 2H, $C\underline{H}_2$-CH); MS (EI) calcd for $C_{11}H_{11}NO_4$, 221.0688; found 221.0693 (48.5%), 116.0626 (100%); Anal. Calcd for $C_{11}H_{11}NO_4$: C, 59.73; H, 5.01; N, 6.33. Found: C, 59.83; H, 5.00; N, 6.21.

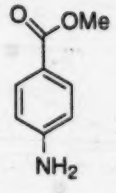
(Carboxymethylmethylene) triphenylphosphorane (51).[75]



Methyl-2-bromoacetate (10 g, 65 mmol) and triphenylphosphine (10 g, 38 mmol) were dissolved in benzene (100 mL) and the solution was heated to reflux and stirred for 24 hours. The mixture was then filtered and the crystalline product was washed with toluene

to give the intermediate phosphonium salt (15 g, 92%). The phophonium salt (14 g, 33 mmol) was dissolved in dioxane/H_2O (150 mL) and sodium hydroxide (1.32 g, 33.0 mmol, in H_2O (10 mL) was added. The solution was stirred for 1 hour and filtered to give an initial crop of **51**. The aqueous layer was extracted with EtOAc (3x50 mL), the organic phase was dried and the solvent was removed. The solid was combined to give the product **51** (7 g, 61%): mp 157-159 °C; IR (KBr disk) 3433 (w, br), 3058 (w), 1618 (s), 1586 (m), 1482 (m) 1441 (s), 1434 (s), 1348 (s), 1121 (s), 884 (s), 747 (s) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) 7.5 (m, 15H, aryl-C<u>H</u>), 3.5 (s, 3H, $CO_2C\underline{H}_3$), 2.9 (s, 1H, $C\underline{H}CO_2CH_3$); MS (EI) calcd for $C_{21}H_{18}O_2P$, 333.1044; found 333.1039; Anal. Calcd for $C_{21}H_{19}O_2P$: C, 75.44; H, 5.73. Found: C, 75.47; H, 5.72.

Methyl 4-Aminobenzoate (53).[68]

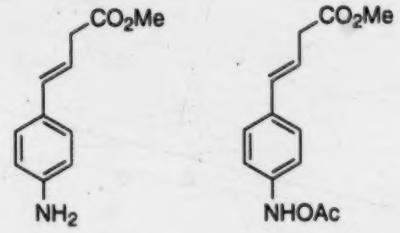


Methyl 4-nitrobenzoate (7.90 g, 43.6 mmol) was dissolved in THF (35 mL) and added to the suspension of $NaBH_2S_3$[37] *via* an addition funnel. The mixture was heated to reflux for 24 hours. The mixture was then diluted with 1 N HCl (50 mL) and Et_2O (50 mL), and the organic layer was washed with H_2O (3x50 mL). The aqueous layer was washed with Et_2O (50 mL), the organic layers were combined, filtered through glass wool, further washed with 1 N HCl (50 mL), H_2O (2x50 mL) and saturated NaCl (50 mL), and then dried (Na_2SO_4). The solvent was removed. The $NaBH_2S_3$ was prepared as follows. Sodium borohydride (1.65 g, 43.6 mmol) and sulfur (4.19 g, 131 mmol) were placed in a dry 250 mL 3 neck round bottom flask, cooled in an ice bath and thoroughly mixed. THF (11 mL)

was rapidly added *via* an addition funnel. The mixture was stirred until there was no further evolution of gas visible, yielding a bright yellow suspension.

The residue was purified by flash chromatography (SiO_2, 25% EtOAc in hexane to 100% EtOAc, R_f 0.4 in 50 % EtOAc in hexane) to give the product **53** (2.2 g, 27%): IR (CHCl3 cast) 3200 (m, br), 1737 (s), 1596 (s), 1345 (s), 1230 (s), 1036 (m), 1108 (m), 857 (m) cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 7.8 (d, J = 7.8 Hz, 2H, aryl-CH ortho to ester), 6.6 (d, J =7.8 Hz, 2H, aryl-CH meta to ester), 4.1 (s, br, 2H, NH_2), 3.9 (s, 3H, CO_2CH_3), MS (EI) calcd for $C_8H_9NO_2$, 151.0633; found 151.0637 (60.55%), 120.1450 (100%).

Methyl 4-(4-Aminophenyl)-3-butenoate (54) and Methyl 4-(4-(*N*-Acetyl) hydroxyaminophenyl)-3-butenoate (55).



54 **55**

Method 1:[37] Methyl 4-nitrophenyl-3-butenoate (**50**) (150 mg, 0.40 mmol) was dissolved in THF (3 mL) and added to $NaBH_2S_3$ (from $NaBH_4$ (26.3 mg, 0.70 mmol) and sulfur (65 mg, 2.0 mmol) that was dissolved in 1 mL THF). The solution was heated to reflux and stirred for 48 hours. It was taken up in saturated $NaHCO_3$ (50 mL) and extracted with EtOAc (3x30 mL). The organic fractions were combined and washed with 1 M HCl (5x20 mL), and the aqueous fractions were brought to pH 8 with saturated $NaHCO_3$ and extracted with EtOAc (3x100 mL). The combined organic layers were dried ($MgSO_4$) and the solvent was removed to give the product **54** (10 mg, 13%) (data given below).

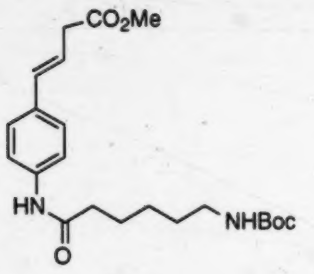
Method 2:[38] Methyl 4-(4-nitrophenyl)-3-butenoate (**50**) (150 mg, 0.68 mmol) was dissolved in EtOAc (30 mL) and $SnCl_2 \cdot 2H_2O$ (766 mg, 3.39 mmol) was added. The solution was heated at reflux for 1 hour then poured into ice (10 mL) and brought to pH 8 with saturated $NaHCO_3$. The mixture was extracted with EtOAc (2x20 mL) and the organic extracts were washed with saturated NaCl (2x20 mL), and dried with Na_2SO_4. The solvent was removed and the residue was purified by flash chromatography (SiO_2, 25% EtOAc in hexane to 100% EtOAc, R_f: 0.5 (**54**) and R_f: 0.4 (**55**). The products were both obtained as oils, **54** (14.1 mg, 10.8%) and **55** (55.6 mg, 35%). Compound **55** was characterized as follows: IR ($CHCl_3$ cast) 3469 (m), 3372 (m), 3232 (w), 3034 (w), 1737 (s), 1683 (m), 1663 (m), 1609 (m), 1581 (s), 1564 (s), 1518 (s) cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 7.2 (m, 2H, aryl C<u>H</u>), 6.6 (m, 4H, aryl C<u>H</u>, C<u>H</u>=), 3.9 (s, br, 1H, N<u>H</u>), 3.7 (s, 3H, $CO_2C\underline{H}_3$), 3.1 (d, J = 7 Hz, 2H, $C\underline{H}_2$), 2.3 (s, 3H, $C\underline{H}_3$); MS (EI) calcd for $C_{13}H_{15}NO_4$, 249.1901; found 249.1991 (22%), 148.0763 (100%).

Method 3:[38] Methyl 4-(4-nitrophenyl)-3-butenoate (**50**) (150 mg, 0.68 mmol) was dissolved in THF (30 mL) and $SnCl_2 \cdot 2H_2O$ (0.7659 g, 3.39 mmol) was added. The solution was heated at reflux for 1 hour, then poured into ice (10 mL) and brought to pH 8 with $NaHCO_3$. The mixture was extracted with EtOAc (2x20 mL), and the organic extracts were washed with saturated NaCl (2x20 mL) and dried with Na_2SO_4. The solvent was removed and the residue was purified by flash chromatography (SiO_2, 10% EtOAc in hexane to 100% EtOAc, R_f: 0.5 (**54**)). The product **54** was obtained as an oil (103 mg, 80%): IR (CH_2Cl_2 cast) 3463 (m), 3373 (m), 3225 (w), 3031 (m), 2950 (m), 2930 (m), 1732 (s), 1622 (s), 1608 (s), 1517 (s), 1436 (m), 1343 (m), 1282 (s), 1260 (s), 1203 (s), 1177 (s), 1108 (m), 967 (m) cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 7.2 (d, J = 8.5 Hz, 2H, aryl C<u>H</u> meta to NH_2), 6.6 (d, J = 8.5 Hz, 2H, aryl C<u>H</u> ortho to NH_2), 6.4 (d, J = 15.6 Hz, 1H, C<u>H</u>=CH), 6.1 (d of t, J = 15.6, 7.1 Hz, 1H, $C\underline{H}CH_2$), 3.7 (s, 3H, $CO_2C\underline{H}_3$), 3.2 (d of d, J = 7.1, 1.2 Hz, 2H, $CHC\underline{H}_2$); MS (EI) calcd for $C_{11}H_{13}NO_2$, 191.0946; found 191.0948 (46%), 132.0819 (100%).

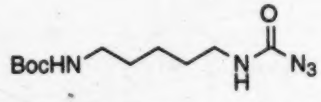
***N*-Boc-6-aminocaproic Acid (56).**

The general procedure for protecting the amine was modified.[39] Aminocaproic acid (3.28 g, 2.50 mmol) was dissolved in dioxane (25 mL) and NaOH (1 M, 25 mL) was added, followed by Boc_2O (6.00 g, 2.75 mmol). The solution was stirred for 2 hours, acidified with $KHSO_4$ (0.4 M) to pH 2 and extracted with EtOAc (3x50 mL). The solvent was dried ($MgSO_4$) and removed. The residue was purified by flash chromatography (SiO_2, 50% EtOAc in hexane to 100% EtOAc, R_f: 0.5 (50% EtOAc)) to yield a white solid (3.58 g, 62%): mp 40-41°C, IR ($CHCl_3$ cast) 3336 (m, br), 3003 (m), 2977 (s), 2936 (s), 2867 (m), 1712 (vs), 1522 (s), 1456 (m), 1437 (m), 1409 (s), 1393 (m), 1367 (s), 1279 (s), 1252 (s) 1170 (s) cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 4.7 (s br, 1H, NH), 3.1 (m, 2H, CH_2NH), 2.4 (t, J = 7.5 Hz, 2H, CH_2CO_2H), 1.7-1.4 (m, 6H, $CH_2CH_2CH_2$), 1.4 (s, 9H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 177 (CO_2H), 156 (-$NHC(O)O$-), 60.3 ($C(CH_3)_3$), 40.2 (CH_2NH), 33.6 (CH_2CO_2H), 29.5 (CH_2), 28.0 ($C(CH_3)_3$), 26.0 (CH_2), 24.2 (CH_2); MS (posCI) $C_{11}H_{21}NO_4$, 231; found 232 (MH^+); Anal. Calcd for $C_{11}H_{21}NO_4$: C, 57.1; N, 6.06; H, 9.15. Found: C, 56.93; N, 6.01; H, 9.44.

Methyl 4-(4-(*N*-Boc-aminohexanoyl)phenyl)-3-butenoate (57) and 1-*N*-(Azido carbonyl)-5-*N*-Boc-1,5-diaminopentane (58).



57



58

Method 1: The general coupling procedure was modified.[40] The amine **54** (100 mg, 0.50 mmol), acid **56** (127 mg, 0.55 mmol, and 4-dimethylaminopyridine (67 mg, 0.55 mmol) were dried separately under high vacuum, DMF (5 mL) was added and the solution was cooled to 0°C. The reaction was initiated by the addition of diphenylphosphoryl azide (118 μL, 0.55 mmol) and triethylamine (76 μL, 0.55 mmol), and the mixture was stirred for 40 hours under Ar. The solvent was removed and the residue was taken up in EtOAc (50 mL). The organic phase was washed with 1 N HCl (2x20 mL), saturated $NaHCO_3$ (2x20 mL), H_2O (20 mL) and saturated NaCl (20 mL). The solution was dried with $MgSO_4$ and the solvent was removed. The residue was purified by flash chromatography (SiO_2, 20% EtOAc in hexane to 100% EtOAc, R_f (**58**) 0.70, Rf (**57**) 0.65 (50% EtOAc). Azido compound **58** was obtained as a yellow oil (50.7 mg, 37%) and product **57** was a white solid (48 mg, 22%) (see data below). Compound **58** was characterized as follows: IR ($CHCl_3$ cast) 3385 (s), 3334 (s), 2983 (m), 2942 (m), 2867 (m), 2171 (m), 2137 (s), 1733 (m), 1699 (s), 1681 (s), 1653 (m), 1559 (m), 1528 (s), 1518 (s), 1476 (m), 1268 (m), 1223 (s), 1189 (m), 1165 (m) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 5.4 (s, br, 1H, NH), 4.6 (s, br, 1H, NH), 3.2 (q, J = 1.3 Hz, 2H, CH_2NHCON_3), 3.1 (m, 2H, CH_2NHCO), 1.6-1.2 (m, 6H, $CH_2CH_2CH_2$), 1.4 (s, 9H,

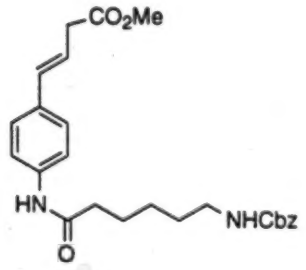
$C\underline{H}_3$); 13C NMR (75 MHz, CD_3OD) δ 159.5, 159.4 ($\underline{C}O$), 41.7, 41.3, 41.2 ($\underline{C}H_2N$, $\underline{C}(CH_3)_3$), 30.6 ($\underline{C}H_2$), 30.0 ($\underline{C}H_2$), 28.8 ($C(\underline{C}H_3)_3$), 24.9 ($\underline{C}H_2$); MS (posCI) 272 (MH+); (EI) calcd for $C_7H_{13}N_5O_3$ 215.1018 found 215.1017 (MH^+-C_4H_9).

Method 2: The general coupling procedure was modified.[42] The amine **54** (120 mg, 0.6 mmol), and acid **56** (180 mg, 0.75 mmol) were dissolved in DMF (5 mL). EEDQ (192 mg, 0.75 mmol) was added and the solution was stirred for 4 days. The solvent was removed and the residue was taken up in EtOAc (50 mL). This was washed with 1 N HCl (2x50 mL), $NaHCO_3$ (saturated solution, 2x50 mL), H_2O (50 mL) and saturated NaCl (50 mL). The solution was dried with $MgSO_4$ and the solvent was removed. The residue was purified by flash chromatography (SiO_2, 20% EtOAc in hexane to 100% EtOAc to give **57** as a white solid (65 mg, 27%) (see data below).

Method 3: The general coupling procedure was modified.[46] The amine **54** (150 mg, 0.78 mmol) and acid fluoride **74** (100 mg, 0.42 mmol) were dissolved in CH_2Cl_2 (50 mL) and 0.1M $NaHCO_3$ (50 mL), and the biphasic solution was stirred for 8 hours. The solvent was removed and the residue was taken up in EtOAc (50 mL). It was then washed with 1 N HCl (2x50 mL), saturated $NaHCO_3$ (2x50 mL), H_2O (50 mL) and saturated NaCl (50 mL). The solution was dried with $MgSO_4$ and the solvent was removed. The residue was purified by flash chromatography (SiO_2, 20% EtOAc in hexane to 100% EtOAc, Rf (**57**) 0.65 (50% EtOAc)). The product **57** was obtained as a white solid (100 mg, 59%): IR (CHCl3 cast) 3319 (m, br), 2975 (m), 2933 (m), 1738 (s), 1735 (s), 1715 (s), 1693 (s), 1684 (s), 1597 (m), 1527 (s), 1516 (s), 1456 (m), 1436 (m), 1410 (m), 1307 (m), 1251 (s), 1167 (s) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.7 (s, br, 1H, N$\underline{H}$-aryl), 7.4 (d, J = 7.9 Hz, 2H, aryl C$\underline{H}$ ortho to amide), 7.2 (d, J = 7.9 Hz, 2H, aryl C$\underline{H}$ meta to amide), 6.9 (d of t, J = 15.9, 7.2 Hz, 0.35H, =C$\underline{H}$$CH_2$), 6.4 (d, J = 15.7 Hz, 0.65H, C$\underline{H}$=), 6.1 (d of t, J = 15.7, 7.1 Hz, 0.65H, =C$\underline{H}$$CH_2$), 5.5 (d of t, J = 15.9, 1.1 Hz, 0.35H, C$\underline{H}$=CH) 4.6 (s, br, 1H, N$\underline{H}$), 3.7 (s, 3H, $CO_2C\underline{H}_3$), 3.2 (d, J = 7.1 Hz, 1.3H, =CHC$\underline{H}_2$), 3.1 (d, J = 7.2 Hz, 0.7H, =CHC$\underline{H}_2$), 3.0 (q, J = 6.4 Hz, 2H,

$CH_2C\underline{H}_2NH$), 2.3 (t, J = 7.4 Hz, 2H, $C\underline{H}_2CO$), 1.8-1.5 (m, 6H, $CH_2C\underline{H}_2C\underline{H}_2$), 1.5 (s, 9H, $C\underline{H}_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.2, 171.5 ($\underline{C}O$), 137.5, 132.8, 130.0, 129.2, 126.7, 120.6, 120.2, 119.8 (aryl $\underline{C}$, vinyl $\underline{C}$), 51.9 ($\underline{C}H_3$ ester), 40.2, 38.1, 37.3, 29.6, 28.4, 26.2, 25.0 (alkyl $\underline{C}$); MS (EI) calcd for $C_{22}H_{32}N_2O_5$, 404.2311; found 404.2309 (4.6%), 191.0941 (100%).

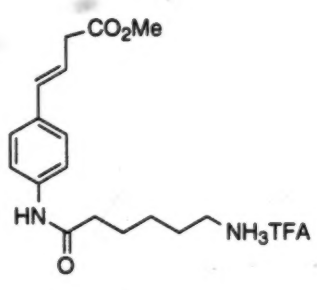
Methyl 4-(4-(*N*-Cbz-6-aminohexanoyl)aminophenyl)-3-butenoate (59).



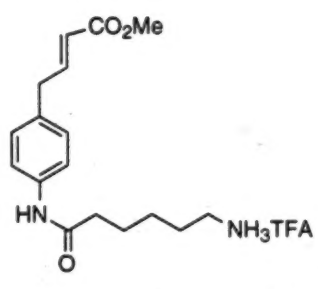
The general coupling procedure was modified.[40] The amine **54** (217 mg, 1.10 mmol), *N*-Cbz-6-aminohexanoic acid (602 mg, 2.30 mmol) and dimethylaminopyridine (138 mg, 1.13 mmol) were dried separately under high vacuum. DMF (20 mL) was added and the solution was cooled to 0°C. The reaction was initiated by the addition of diphenylphosphoryl azide (489 μL, 1.1 mmol) and triethylamine (152 μL, 1.1 mmol) and the mixture was stirred for 18 hours under Ar. The solvent was removed and the residue was taken up in EtOAc (50 mL). This was washed with 1 N HCl (2x20 mL), saturated $NaHCO_3$ (2x20 mL), H_2O (20 mL) and saturated NaCl (20 mL). The solution was dried with $MgSO_4$ and the solvent was removed. The residue was purified by flash chromatography (SiO_2, 20% EtOAc in hexane to 100% EtOAc, R_f (**59**) 0.6 in 50% EtOAc). Product **59** was obtained as a white solid (104 mg, 21%): IR ($CHCl_3$ cast) 3317 (s), 2951 (m), 2928 (m), 2921 (m), 2852 (m), 1739 (m), 1718 (m), 1690 (s), 1655 (m), 1636 (w), 1532 (m), 1279 (m), 1264 (m), 1197 (m), 1166 (m), 1142 (m), 697 (m) cm^{-1};

^{1}H NMR (200 MHz, $CDCl_3$) δ 7.5 (d, *J* = 8.7 Hz, 2H, arylC<u>H</u>), 7.3 (m, 8H, arylC<u>H</u>), 6.5 (d, *J* = 15.9 Hz, 1H, C<u>H</u>=CH), 6.2 (d of t, *J* = 15.9, 6.9 Hz, 1H, CH=C<u>H</u>CH_2), 5.1 (s, 2H, $C\underline{H}_2$benzyl ester), 4.9 (s, br, N<u>H</u>), 3.8 (s, 3H, $CO_2C\underline{H}_3$), 3.2 (m, 4H, $CH{=}CHC\underline{H}_2$, $C\underline{H}_2NH$), 2.4 (t, *J* = 7.4 Hz, 2H, $C\underline{H}_2CO$), 1.7-1.4 (m, 6H, $C\underline{H}_2C\underline{H}_2C\underline{H}_2$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.2 (<u>C</u>O ester), 166.9 (<u>C</u>O amide), 156.5 (<u>C</u>O), 147.6, 136.7, 136.6, 133.3, 129.2, 128.5, 128.0, 127.9, 121.8, 120.1 (aryl, vinyl <u>C</u>), 66.6 ($\underline{C}H_2$ benzyl), 51.4 ($\underline{C}H_3$ ester), 40.7 ($\underline{C}H_2N$), 37.9 ($CH{=}CH\underline{C}H_2$), 37.3 ($\underline{C}H_2CO$), 29.6, 26.1, 24.9 ($\underline{C}H_2$); MS (EI) calcd for $C_{25}H_{30}N_2O_5$, 438.2154; found 438.2152 (3.7%), 132.0816 (100%).

Trifluoroacetate Salts of Methyl 4-(4-(6-Aminohexanoyl)amino)phenyl)-3-butenoate (60a) and Methyl 4-(4-(6-Aminohexanoyl)amino)phenyl)-2-butenoate (60b)



60a

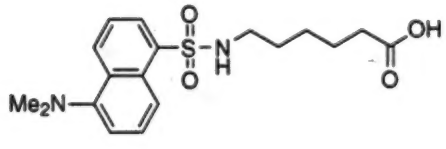


60b

Compound **57** (60 mg, 0.14 mmol) was dissolved in TFA (3 mL) and stirred for 1 hour. The solvent was removed to give a 1:1 mixture of the products **60a** and **60b** as an oil (62 mg, 95%): IR (MeOH cast) 3028 (s, br), 2947 (s, br), 1717 (s), 1661 (s), 1596 (s), 1529 (s), 1411 (m), 1310 (m), 1257 (m), 1176 (m) cm^{-1}; ^{1}H NMR (300 MHz, CD_3OD, **60a**) δ 7.5 (m, 2H, aryl-C<u>H</u>), 7.3 (d, *J* = 7.8 Hz, 1.4H, aryl-C<u>H</u>), 7.2 (d, *J* = 7.8 Hz, 0.6H, aryl-C<u>H</u>), 6.5 (d, *J* = 15.9 Hz, 1H, C<u>H</u>=CHCH_2), 6.2 (d of t, *J* = 15.9, 6.6 Hz, 0.7H,

C<u>H</u>=CHCH$_2$), 3.8 (s, 0.9H, CO$_2$C<u>H</u>$_3$), 3.7 (s, 2.1H, CO$_2$C<u>H</u>$_3$), 3.3 (d, *J* = 6.6 Hz, 1.4H, CH=CHC<u>H</u>$_2$), 3.0 (t, *J* = 7.2 Hz, 2H, C<u>H</u>$_2$N), 2.4 (t, *J* = 7.5 Hz, 2H, C<u>H</u>$_2$CO), 1.7 (m, 4H, CH$_2$C<u>H</u>$_2$C<u>H</u>$_2$), 1.5 (m, 2H, CH$_2$C<u>H</u>$_2$CH$_2$); ^{1}H NMR (300 MHz, CD$_3$OD, **60b**) δ 7.5 (m, 2H, aryl-C<u>H</u>), 7.3 (d, *J* = 7.8 Hz, 1.4H, aryl-C<u>H</u>), 7.2 (d, *J* = 7.8 Hz, 0.6H, aryl-C<u>H</u>), 7.1 (d of t, *J* = 16, 6.6 Hz, 1H, CH=C<u>H</u>CH$_2$), 5.8 (d of t, *J* = 16, 2.8 Hz, 1H, C<u>H</u>=CHCH$_2$), 3.8 (s, 0.9H, CO$_2$C<u>H</u>$_3$), 3.7 (s, 2.1H, CO$_2$C<u>H</u>$_3$), 3.5 (d, *J* = 6.6 Hz, 2H, CH=CHC<u>H</u>$_2$), 3.0 (t, *J* = 7.2 Hz, 2H, C<u>H</u>$_2$N), 2.4 (t, *J* = 7.5 Hz, 2H, C<u>H</u>$_2$CO), 1.7 (m, 4H, CH$_2$C<u>H</u>$_2$C<u>H</u>$_2$), 1.5 (m, 2H, CH$_2$C<u>H</u>$_2$CH$_2$); ^{13}C NMR (100 MHz, CDCl$_3$) δ 177.6 (<u>C</u>O ester), 168.6 (<u>C</u>O amide), 140.2, 139.3, 135.2, 134.3, 131.8, 130.0, 127.6, 121.6 (aryl, vinyl <u>C</u>), 40.5 (<u>C</u>H$_2$N), 38.6 (<u>C</u>H$_2$CH=CH), 37.4 (<u>C</u>H$_2$CO), 28.3, 27.0, 26.0 (<u>C</u>H$_2$); MS (EI) calcd for C$_{17}$H$_{25}$N$_2$O$_3$, 304.1787; found 304.1768 (3%), 132.0812 (100%).

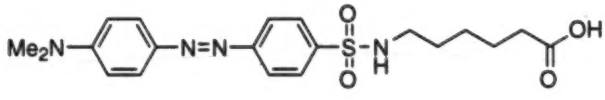
6-(*N*-Dansylamino)hexanoic Acid (62).



The general procedure was modified.[45] 6-Aminohexanoic acid (200 mg, 1.5 mmol) was dissolved in carbonate buffer (0.1M NaHCO$_3$ /Na$_2$CO$_3$, pH 8.5, 200 mL). Dansyl chloride (445 mg, 1.65 mmol) in 200 mL of acetone was added to the carbonate buffer solution, and the mixture was stirred until the yellow colour faded (about 8 hours). The pH of the solution was adjusted to 2 with 1 M HCl. The solution was extracted with EtOAc (3x100 mL), the organic phase was dried with MgSO$_4$, and the solvent was removed. A yellow solid **62** (312 mg, 57%) was obtained: IR (μscope) 3303 (m), 2940 (m), 2863 (m), 1703 (m), 1574 (w), 1455 (m), 1431 (m), 1313 (s), 1143 (s), 1095 (m),

790 (m) cm^{-1}; ^1H NMR (200 MHz, CD_3OD) δ 8.5 (d of t, *J* = 8.5, 1.0, 1H, aryl-C<u>H</u> ortho to SO_2), 8.4 (d, *J* = 9 Hz, 1H, aryl-C<u>H</u> para to NMe_2), 8.2 (d of d, *J* = 7.3, 1.3 Hz, 1H, aryl-C<u>H</u> para to SO_2), 7.5 (m, 2H, aryl-C<u>H</u> meta to NMe_2, meta to SO_2), 7.2 (d of d, *J* = 8.5, 0.8 Hz, 1H, ortho to NMe_2), 2.8 (t, *J* = 5.2 Hz, 2H, NHC<u>H</u>$_2$), 2.8 (s, 6H, N(C<u>H</u>$_3$)$_2$), 2.0 (m, 2H, C<u>H</u>$_2$CO$_2$H), 1.4-1.1 (m, 6H, $CH_2C\underline{H}_2CH_2$); ^{13}C NMR (75 MHz, CD_3OD) δ 176.2 (<u>C</u>O), 150.5, 137.3, 130.8, 130.5, 130.4, 130.2, 129.0, 124.7, 121.5, 116.9 (aryl <u>C</u>), 51.9, 46.0, 43.5, 34.5, 30.0, 26.8, 25.2 (alkyl <u>C</u>); MS (posFAB) $C_{18}H_{24}N_2O_4S$, 364.1457; found 365.1 (35%), 170.1 (100%).

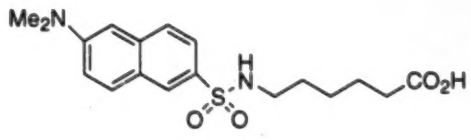
6-(*N*-Dabsylamino)hexanoic Acid (64).



The general procedure was modified.[45] 6-Aminohexanoic acid (89 mg, 0.68 mmol) was dissolved in carbonate buffer (0.1 M $NaHCO_3/Na_2CO_3$, pH 8.5, 100 mL). Dabsyl chloride (200 mg, 0.618 mmol) in 100 mL of acetone was added to the carbonate buffer solution and the mixture was stirred at 60°C for 30 minutes. The pH of the solution was adjusted to 2 with 1 M HCl. The mixture was extracted with EtOAc (3x100 mL), and the organic phase was dried with $MgSO_4$ and the solvent was removed. A red solid **64** (249 mg, 96%) was obtained: mp 151-153 °C; IR (μscope) 2943 (m, br), 1708 (s), 1605 (s), 1424 (m), 1325 (m), 1255 (m), 1142 (s), 1087 (m), 845 (m) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.9 (d, *J* = 8 Hz, 2H, aryl-C<u>H</u> ortho to SO_2), 7.8 (m, 4H, ortho and meta to N_2), 6.8 (d, *J* = 8.0 Hz, 2H, ortho to NMe_2), 5.2 (br t, *J* = 4.8 Hz, 1H, N<u>H</u>), 3.0 (q, *J* = 4.8 Hz, 2H, C<u>H</u>$_2$NH), 2.9 (s, 6H, N(C<u>H</u>$_3$)$_2$), 2.3 (t, *J* = 6.4 Hz, 2H, C<u>H</u>$_2$CO$_2$H), 1.6-1.4 (m, 6H, $CH_2C\underline{H}_2CH_2$); ^{13}C NMR (75 MHz, CD_3OD) δ 174.2 (<u>C</u>O), 154.9, 144.7,

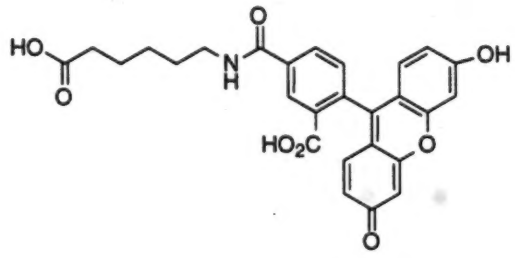
141.7, 129.0, 126.8, 123.4, 128.0, 112.7 (aryl C), 52.0, 43.9, 40.4, 30.4, 27.2, 25.6 (alkyl C); MS (posFAB) $C_{20}H_{26}N_4O_4S$, 418.1675; found 418.9 (47%), 84.9 (100%).

6-((6-Dimethylamino-2-naphthalenesulfonyl)amino)hexanoic Acid (66).



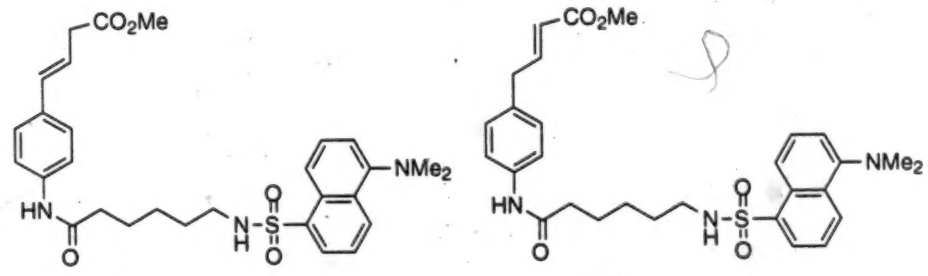
The general procedure was modified.[45] 6-Aminohexanoic acid (125 mg, 0.90 mmol) was dissolved in carbonate buffer (0.1 M $NaHCO_3/Na_2CO_3$, pH 8.5, 100 mL). A solution of 6-dimethylamino-2-napthalenesulfonyl chloride (250 mg, 0.93 mmol) in 100 mL of DMF was added and the mixture was stirred at room temperature for 4 hours. The pH of the solution was adjusted to 2 with 1 M HCl, and it was extracted with EtOAc (3x100 mL). The organic phase was dried with $MgSO_4$ and the solvent was removed. Compound **66** was isolated as a yellow solid (322 mg, 98%): ^{1}H NMR (300 MHz, CD_3OD) δ 8.2 (d, J = 1.5Hz, 1H, aryl-CH ortho to SO_2), 7.8 (d, J = 9.2 Hz, 1H, aryl-CH meta to NMe_2), 7.8 (d, J = 8.8 Hz, 1H, aryl-CH ortho to SO_2), 7.6 (d of d, J = 8.8, 2.1 Hz, 1H, aryl-CH meta to SO_2), 7.3 (d of d, J = 9.2, 2.5 Hz, 1H, aryl-CH ortho to NMe_2), 7.00 (d, J = 2.1 Hz, 1H, aryl-CH ortho to NMe_2), 3.0 (s, 6H, $N(CH_3)_2$), 2.8 (t, J = 6.8 Hz, 2H, CH_2N), 2.1 (t, 7.5 Hz, 2H, CH_2CO), 1.5 (m, 4H, $CH_2CH_2CH_2$), 1.4 (m, 2H, $CH_2CH_2CH_2$); MS (posFAB) $C_{18}H_{24}N_2O_4S$, 364.1; found 365 (MH^+, 32%), 170.1 (100%).

6-(*N*-(Fluorescein-5-carboxylic acid)amino)hexanoic Acid (68).



The general procedure was modified.[45] 6-Aminohexanoic acid (50 mg, 0.36 mmol) was dissolved in carbonate buffer (0.1 M $NaHCO_3/Na_2CO_3$, pH 8.5, 100 mL). A solution of fluorescein-5-carboxylic acid-*N*-hydroxysuccinic ester (20 mg, 0.04 mmol) in DMF (100 mL) was added and the mixture was stirred at room temperature for 4 hours. The pH of the solution was adjusted to 2 with 1 M HCl and the solution was extracted with EtOAc (3x100 mL). The organic extracts were dried with $MgSO_4$ and the solvent was removed. The product was obtained as a yellow solid **68** (23 mg, 98%): IR (MeOH cast) 3300 (m, br), 1651 (s), 1615 (s), 1591 (s), 1463 (m), 1384 (m), 1110 (m) cm^{-1}; ^1H NMR (300 MHz, CD_3OD) δ 8.5 (d, J = 1.2 Hz, 1H, aryl-C<u>H</u> ortho to CO_2H), 8.1 (d of d, J = 8.6, 1.2 Hz, 1H, aryl-C<u>H</u> para to CO_2H), 7.2 (d, J = 8.6 Hz, 1H, aryl-C<u>H</u> meta to CO_2H), 6.8 (s, 2H, aryl-C<u>H</u> ortho to OH, vinyl <u>H</u> α to ketone), 6.7-6.6 (m, 4H, aryl-C<u>H</u> ortho and meta to hydroxyl, vinyl <u>H</u> α and β to ketone), 3.6 (m, J = 7.3 Hz, 2H, $C\underline{H}_2N$) 2.4 (t, J = 7.3, 2H, $C\underline{H}_2CO$), 1.7 (m, 4H, $CH_2C\underline{H}_2CH_2$), 1.5 (m, 2H, $CH_2C\underline{H}_2CH_2$); MS (posFAB) $C_{27}H_{23}NO_8$, 489.1; found 489.8 (0.8%), 118.9 (100%).

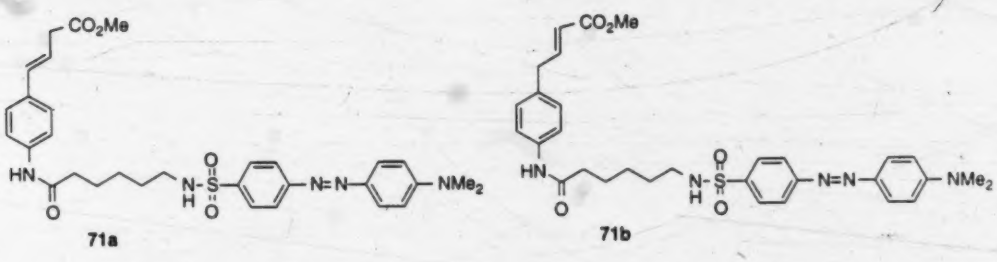
Methyl 4-[4-[*N*-(6-((*N*-dansyl)amino)hexanoyl)amino]phenyl]-3-butenoate (69a) and Methyl 4-[4-[*N*-(6-((*N*-dansyl)amino)hexanoyl)amino] phenyl]-2-butenoate (69b).



The general coupling procedure was modified.[40] Amine **54** (100 mg, 0.5 mmol), *N*-dansyl-6-aminohexanoic acid **62** (200 mg, 0.55 mmol) and dimethylaminopyridine (67 mg, 0.55 mmol) were dried under high vacuum, DMF (20 mL) was added and the solution was cooled to 0°C. The reaction was initiated by the addition of diphenylphosphoryl azide (118 µL, 0.55 mmol) and triethylamine (76 µL, 0.55 mmol). The mixture was stirred for 48 hours under Ar. The solvent was removed and the residue was taken up in EtOAc (50 mL) and then washed with 1 N HCl (2x20 mL), saturated $NaHCO_3$ (2x20 mL), H_2O (20 mL) and saturated NaCl (20 mL). The solution was dried with $MgSO_4$ and the solvent was removed. The residue was purified by flash chromatography (SiO_2, 25% EtOAc in hexane to 100% EtOAc, R_f (**69**) 0.3 (in EtOAc)). The product was obtained as a 1:1 mixture of **69 a** and **b** as a yellow oil (39 mg, 26%): HPLC (Novapak, RP C-18, monitored at 254 nm); solvent A: 0.1% TFA in water, solvent B 80/20/0.1 MeCN/$H_2$0/TFA; starting conditions 70/30 A/B changing to 40/60 A/B over 20 minutes, holding for 5 minutes, reverting to 70/30 A/B in 5 minutes and holding for 5 minutes retention time of 16.5 minutes; IR ($CHCl_3$ cast) 3319 (s, br), 3112 (m), 3017 (m), 2960 (s), 2930 (s), 2860 (m), 2790 (w), 2140 (w), 1731 (s), 1687 (m), 1673 (m), 1592 (s), 1528 (s), 1515 (s), 1410 (m), 1313

(s), 1260 (s), 1160 (s), 1019 (s), 794 (s), 757 (s) cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$, **69a**) δ 8.4 (d, *J* = 8.5 Hz, 1H, aryl-C<u>H</u> ortho to SO_2), 8.3 (d, *J* = 8.5 Hz, 1H aryl-C<u>H</u> meta to NMe_2), 8.1 (d of d, *J* = 7.3, 1.2 Hz, aryl-C<u>H</u> meta to NMe_2), 7.9 (d, *J* = 9 Hz, N<u>H</u>CO), 7.4 (m, 3H, aryl-C<u>H</u> meta, ortho to SO_2, ortho to NMe_2), 7.1 (m, 4H, aryl-C<u>H</u> ortho and meta to NH), 6.4 (d, *J* = 15.9 Hz, 1H, C<u>H</u>=CHCH$_2$), 6.1 (d of t, *J* = 15.9, 6.9 Hz, 1H, CH=C<u>H</u>CH$_2$), 5.4 (br t, *J* = 5.7 Hz, 1H, N<u>H</u>SO_2), 3.7 (s, 3H, $CO_2C\underline{H}_3$), 3.2 (d, *J* = 6.7 Hz, 1H, C<u>H</u>$_2$CH=CH), 2.8 (m, 8H, N(C<u>H</u>$_3$)$_2$, C<u>H</u>$_2$NH), 2.2 (t, *J* = 7.6 Hz, 2H, C<u>H</u>$_2$CO), 1.5-1.1 (m, 6H, $CH_2C\underline{H}_2CH_2$); ^1H NMR (200 MHz, $CDCl_3$) δ 8.4 (d, *J* = 8.5 Hz, 1H, aryl-C<u>H</u> ortho to SO_2), 8.3 (d, *J* = 8.5 Hz, 1H aryl-C<u>H</u> meta to NMe_2), 8.1 (d of d, *J* = 7.3, 1.2 Hz, aryl-C<u>H</u> meta to NMe_2), 7.9 (d, *J* = 9 Hz, N<u>H</u>CO), 7.4 (m, 3H, aryl-C<u>H</u> meta, ortho to SO_2, ortho to NMe_2), 7.1 (m, 5 H, aryl-C<u>H</u> ortho and meta to NH, C<u>H</u>=CHCO_2Me), 5.7 (d of t, *J* = 14.1, 1.5 Hz, 0.5H, CH=C<u>H</u>CO_2Me), 5.4 (br t, *J* = 5.7 Hz, 1H, N<u>H</u>SO_2), 3.7 (s, 3H, $CO_2C\underline{H}_3$), 3.4 (d, *J* = 5.9 Hz, 1H, C<u>H</u>$_2$CH=CH), 2.8 (m, 8H, N(C<u>H</u>$_3$)$_2$, C<u>H</u>$_2$NH), 2.2 (t, *J* = 7.6 Hz, 2H, C<u>H</u>$_2$CO), 1.5-1.1 (m, 6H, $CH_2C\underline{H}_2CH_2$);MS (EI) calcd for $C_{29}H_{35}N_3O_5S$, 537.2297; found 537.2297 (14.7%), 171.1.45 (100%).

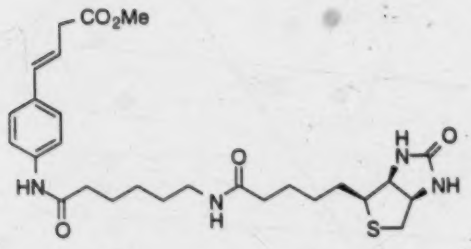
Methyl 4-[4-[*N*-(6-((*N*-dabsyl)amino)hexanoyl)amino]phenyl]-3-butenoate (71a) and Methyl 4-[4-[*N*-(6-((*N*-dabsyl)amino)hexanoyl)amino] phenyl]-2-butenoate (71b).



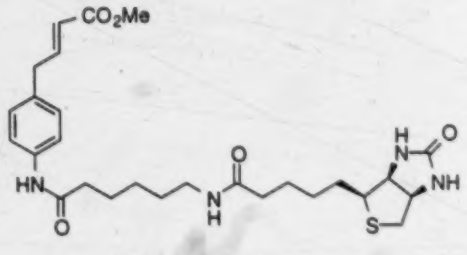
The literature procedure was modified.[30] Amine (**54**) (82 mg, 0.40 mmol), and *N*-dabsyl-6-aminohexanoic acid (**64**) (40 mg, 0.44 mmol), were dried separately under high vacuum. DMF (10 mL) was added and the solution was cooled to 0°C. The reaction was initiated by the addition of BOP reagent (20 mg, 0.44 mmol) and triethylamine (60 μL, 0.44 mmol) and the mixture was stirred for 72 hours under Ar. The solvent was removed and the residue was taken up in EtOAc (50 mL) and then washed with 1 N HCl (2x20 mL), saturated $NaHCO_3$ (2x20 mL), H_2O (20 mL) and saturated NaCl (20 mL). The solution was dried with $MgSO_4$ and the solvent was removed. The residue was purified by flash chromatography (SiO_2, 25% EtOAc in hexane to 100% EtOAc, Rf (**71**) 0.2 (in EtOAc)). Product **71** was obtained an oil (24 mg, 20%) as a 1:1 mixture of **71a** and **71b**: HPLC (Novapak, RP C-18, monitored at 254 nm); solvent A: 0.1% TFA in water, solvent B 80/20/0.1 MeCN/$H_2$0/TFA; starting conditions 70/30 A/B changing to 40/60 A/B over 20 minutes, holding for 5 minutes, reverting to 70/30 A/B in 5 minutes and holding for 5 minutes; retention time of 18.0 minutes: IR ($CHCl_3$ cast) 2957 (m), 2923 (m), 1738 (s), 1693 (m), 1463 (m), 1260 (m), 1134 (m), 1092 (m), 799 (m) cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) δ 7.9 (m, 6H, aryl-C<u>H</u>), 7.4 (m, 3H, aryl-C<u>H</u>), 7.2 (m, 3H, aryl-C<u>H</u>, N<u>H</u>CO), 7.0 (m, 2H, aryl-C<u>H</u>), 6.7 (d, J = 8 Hz, 2H, aryl-C<u>H</u>), 6.3 (d, J = 15.9 Hz, 1H, C<u>H</u>=CHCH$_2$), 6.1 (d of t, J = 15.9, 6.8 Hz, 1H, CH=C<u>H</u>CH$_2$), 4.9 (m, 1H, N<u>H</u>SO$_2$),

3.7 (s, 3H, $CO_2C\underline{H}_3$), 3.1 (s, 6H, $N(C\underline{H}_3)_2$), 3.0 (m, 3H, $C\underline{H}_2CH{=}CH$, $C\underline{H}_2N$), 2.2 (m, 2H, $C\underline{H}_2CO$), 1.5-1.2 (m, 6H, $CH_2C\underline{H}_2CH_2$); ^{1}H NMR (360 MHz, $CDCl_3$) δ 7.9 (m, 6H, aryl-C$\underline{H}$), 7.4 (m, 3H, aryl-C$\underline{H}$), 7.2 (m, 3H, aryl-C$\underline{H}$, N$\underline{H}$CO), 7.0 (m, 3H, aryl-C$\underline{H}$, C$\underline{H}$=CH), 6.7 (d, J = 8 Hz, 2H, aryl-C$\underline{H}$), 15.7 (d, J = 15.7 Hz, 0.5H, C$\underline{H}$=$CHCH_2$), 4.9 (m, 1H, N$\underline{H}SO_2$), 3.7 (s, 3H, $CO_2C\underline{H}_3$), 3.2 (m, 1H, $C\underline{H}_2CH{=}CH$), 3.1 (s, 6H, $N(C\underline{H}_3)_2$), 3.0 (m, 3H, $C\underline{H}_2CH{=}CH$, $C\underline{H}_2N$), 2.2 (m, 2H, $C\underline{H}_2CO$), 1.5-1.2 (m, 6H, $CH_2C\underline{H}_2CH_2$); MS (posFAB) $C_{31}H_{37}N_5O_5S$, 591.3; found 591.8 (0.6%), 118.9 (100%).

Methyl 4-[4-[*N*-(6-((*N*-biotin)amino)hexanoyl)amino]phenyl]-3-butenoate (72a) and Methyl 4-[4-[*N*-(6-((*N*-biotin)amino)hexanoyl)amino] phenyl]-2-butenoate (72b).



72a

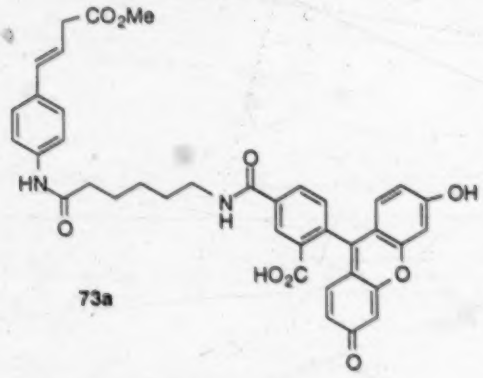


72b

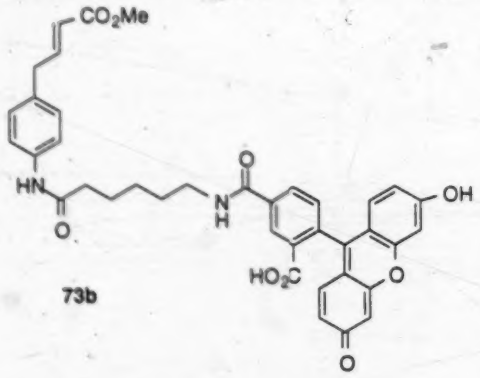
The general coupling procedure was modified.[26] Amine **54** (250 mg, 1.3 mmol) and *N*-biotin-6-aminohexanoic acid (100 mg, 0.23 mmol) were dried under high vacuum, DMF (50 mL) was added and the solution was cooled to 0°C. The reaction was initiated by the addition of BOP reagent (100 mg, 0.23 mmol) and triethylamine (20 μL, 0.23 mmol), and the mixture was stirred for 72 hours under Ar. The solvent was removed, and the residue was taken up in EtOAc (50 mL) and then washed with 1 N HCl (2x20 mL), saturated $NaHCO_3$ (2x20 mL), H_2O (20 mL) and saturated NaCl (20 mL). The solution was dried with $MgSO_4$ and the solvent was removed. The residue was purified by reverse phase HPLC (Novapak, RP C-18, monitored at 254 nm); solvent A: 0.1% TFA in water, solvent B 80/20/0.1 MeCN/$H_2$0/TFA; starting conditions 70/30 A/B changing to 40/60 A/B over 20 minutes, holding for 5 minutes, reverting to 70/30 A/B in 5 minutes and holding for 5 minutes. The product was an 11:1 mixture of **72a** and **b** (72 mg, 21%) and had a retention time of 9.8 minutes: IR (MeOH cast) 3287 (m, br), 2928 (m), 1695 (s), 1651 (s), 1644 (s), 1599 (m), 1531 (s), 1204 (m), 1167 (m) cm^{-1}; ^1H NMR (300 MHz, D_2O, **72a**) δ 7.5 (d, J = 8.4 Hz, 2H, aryl-C<u>H</u> ortho to NH), 7.3 (d, J = 8.4 Hz, 2H, aryl-C<u>H</u> meta to NH), 6.5, (d, J = 15.9 Hz, 1H, C<u>H</u>=CHCH$_2$), 6.2 (d of t, J = 15.9, 7.0 Hz, 1H, CH=C<u>H</u>CH$_2$), 4.5 (m, 1H, SCH$_2$C<u>H</u>), 4.3 (m, 1H, SCHRC<u>H</u>), 3.7 (s, 3H, CO_2CH_3),

3.2 (d, J = 7.0 Hz, 2H, CH=CHC$\underline{H}_2$), 3.2 (t, J = 6.7 Hz, 2H, C$\underline{H}_2$N), 2.9 (d of d, J = 12.8, 4.8 Hz, 1H, SC$\underline{H}$HCH), 2.7 (d, J = 12.8, 1H, SCH$\underline{H}$CH), 2.4 (t, J = 7.2 Hz, 2H, C$\underline{H}_2$CONH aryl), 2.2 (t, J = 7.3 Hz, 2H, C$\underline{H}_2$CONHbiotin), 1.8-1.2 (m, 12H, CH$_2$C$\underline{H}_2$CH$_2$); ^{13}C NMR (125 MHz, CD_3OD), HMQC, HMBC) δ 175.8 (NH$\underline{C}$OCH$_2$biotin), 174.1 (arylNH$\underline{C}$OCH$_2$), 173.6 ($\underline{C}$O$_2$Me), 166.0 (NH$\underline{C}$ONH), 139.3 (ipso $\underline{C}$ amide), 134.2 (ipso $\underline{C}$ vinyl), 133.9 ($\underline{C}$H=CHCH$_2$), 127.7 ($\underline{C}$-ortho to vinyl group) 121.9 (CH=$\underline{C}$HCH$_2$), 121.2 ($\underline{C}$-ortho to amide), 63.4 ($\underline{C}$HCHRS), 61.6 ($\underline{C}$HCH$_2$S), 56.9 ($\underline{C}$HRS), 52.3 (CO$_2\underline{C}$H3), 41.0 ($\underline{C}$H$_2$S), 40.1 ($\underline{C}$H$_2$NH), 38.7 (CH=CH$\underline{C}$H$_2$), 37.8 (arylNHCO$\underline{C}$H$_2$), 36.8 (NHCO$\underline{C}$H$_2$biotin), 30.1 (SCHR$\underline{C}$H$_2$), 29.7 (alkylNHCH$_2\underline{C}$H$_2$), 29.4 (biotinCOCH$_2\underline{C}$H$_2$), 27.5 (alkylNHCH$_2$ CH$_2\underline{C}$H$_2$), 26.9 (SCHRCH$_2\underline{C}$H$_2$), 26.4 (arylNHCOCH$_2\underline{C}$H$_2$); MS (posFAB) $C_{27}H_{38}N_4O_5S$, 530; found 553 (M+Na).

Methyl 4-[4-[*N*-(6-((*N*-fluorescein-5-carboxylic acid)amino) hexan-oyl)amino] phenyl]-3-butenoate (73a) and Methyl 4-[4-[*N*-(6-((*N*-fluorescein-5-carboxylic acid) amino)hexanoyl)amino]phenyl]-2-butenoate (73b).



73a

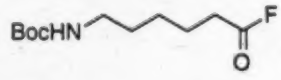


73b

The general procedure was modified.[47] A methyl ester (**60**) (18 mg, 0.047 mmol) was dissolved in carbonate buffer (0.1 M $NaHCO_3/Na_2CO_3$, pH 8.5, 100 mL). A solution of fluorescein *N*-hydroxy succinimic ester (20 mg, 0.04 mmol) in DMF (100 mL) was added

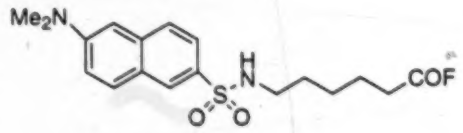
to the carbonate buffer solution, and the mixture was stirred at room temperature for 4 hours. The pH was adjusted to 2 with 1 M HCl, and the solution was extracted with EtOAc (3x100 mL). The organic extracts were dried with $MgSO_4$ and the solvent was removed. The product was obtained as a yellow solid (25 mg, 84%) as a 3:1 mixture of **73 a** and **b**: HPLC (Novapak, RP C-18, monitored at 254 nm); solvent A: 0.1% TFA in water, solvent B 80/20/0.1 MeCN/H_20/TFA; starting conditions 70/30 A/B changing to 40/60 A/B over 20 minutes, holding for 5 minutes, reverting to 70/30 A/B in 5 minutes and holding for 5 minutes; retention time of 9.1 minutes: IR (MeOH cast) 3331 (m, br), 2918 (m), 1694 (m), 1683 (s), 1615 (s), 1575 (s), 1538 (m), 1455 (m), 1330 (s), 1137 (m) cm^{-1}; ^1H NMR (300 MHz, CD_3OD, **73a**) δ 8.5 (d, *J* = 1.2 Hz, 1H, aryl-C<u>H</u> ortho to CO_2H), 8.1 (d of d, *J* = 8.6, 1.2 Hz, 1H, aryl-C<u>H</u> para to CO_2H), 7.5 (d, *J* = 9.4 Hz, 2H, aryl-C<u>H</u> ortho to NHCO), 7.3 (d, *J* = 9.4 Hz, 2H, aryl-C<u>H</u> para to NHCO), 7.2 (d, *J* = 8.6 Hz, 1H, aryl-C<u>H</u> meta to CO_2H), 6.8 (s, 2H, aryl-C<u>H</u> ortho to OH, vinyl <u>H</u> α to ketone), 6.7-6.6 (m, 4H, aryl-C<u>H</u> ortho and meta to hydroxyl, vinyl <u>H</u> α and β to ketone,), 6.4 (d, *J* = 17.3 Hz, 1H, C<u>H</u>=CHCH$_2$), 6.2 (d of d, *J* = 17.3, 7.3 Hz, 1H, CH=C<u>H</u>CH$_2$), 3.6 (s, 3H, $CO_2C\underline{H}_3$), 3.5 (t, *J* = 7.3 Hz, 2H, C<u>H</u>$_2$N), 3.2 (d of d, *J* = 8.6, 1.4, 2H, CH=CHC<u>H</u>$_2$), 2.4 (t, *J* = 7.3, 2H, C<u>H</u>$_2$CO), 1.8 (m, 4H, $CH_2C\underline{H}_2CH_2$), 1.6 (m, 2H, $CH_2C\underline{H}_2CH_2$); ^1H NMR (300 MHz, CD_3OD, **73b**) δ 8.5 (d, *J* = 1.2 Hz, 1H, aryl-C<u>H</u> ortho to CO_2H), 8.1 (d of d, *J* = 8.6, 1.2 Hz, 1H, aryl-C<u>H</u> para to CO_2H), 7.5 (d, *J* = 9.4 Hz, 2H, aryl-C<u>H</u> ortho to NHCO), 7.3 (d, *J* = 9.4 Hz, 2H, aryl-C<u>H</u> para to NHCO), 7.2 (d, *J* = 8.6 Hz, 1H, aryl-C<u>H</u> meta to CO_2H), 6.8 (s, 2H, aryl-C<u>H</u> ortho to OH, vinyl <u>H</u> α to ketone), 6.7-6.6 (m, 5H, aryl-C<u>H</u> ortho and meta to hydroxyl, vinyl <u>H</u> α and β to ketone, $CH_2C\underline{H}$=CH), 5.7 (d, *J* = 16.5 Hz, 1H, CH_2CH=C<u>H</u>), 3.6 (s, 3H, $CO_2C\underline{H}_3$), 3.5 (t, *J* = 7.3 Hz, 2H, C<u>H</u>$_2$N), 3.1 (d, *J* = 7.6, 2H, CH=CHC<u>H</u>$_2$), 2.4 (t, *J* = 7.3, 2H, C<u>H</u>$_2$CO), 1.8 (m, 4H, $CH_2C\underline{H}_2CH_2$), 1.6 (m, 2H, $CH_2C\underline{H}_2CH_2$); MS (posFAB) $C_{37}H_{34}N_2O_9$, 650.2; found 696 (0.4%, M+2Na$^+$) 119.0 (100%).

6-(*N*-Boc-amino)hexanoyl Fluoride (74).



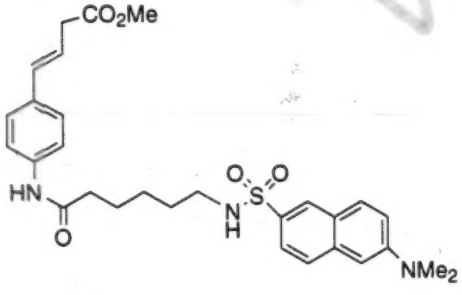
The general literature procedure was modified.[46] 6-(*N*-Boc-amino)hexanoic acid **56** (200 mg, 0.86 mmol) was dissolved in CH_2Cl_2 (75 mL), cyanuric fluoride (174 mg, 1.3 mmol) and pyridine (50 μL, 0.50 mmol) were added. The solution was cooled to 0°C and stirred for 3 hours. The solution was then washed with H_2O (50 mL) and 1 M HCl (50 mL). The organic phase was dried ($MgSO_4$) and the solvent was removed. The product **74** (160 mg, 81%)was obtained as an oil: IR ($CHCl_3$ cast) 2976 (br, m), 2934 (br, s), 1711 (s), 1521 (m), 1408 (m), 1366 (s), 1169 (s) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 4.6 (s br, 1H, NH), 3.1 (t, J = 6.8 Hz, 2H, CH_2N), 2.5 (t, J = 6.8 Hz, 2H, CH_2CO), 1.7 (m, 2H, $CH_2CH_2CH_2$), 1.4 (m, 4H, $CH_2CH_2CH_2$), 1.4 (s, 9H, $C(CH_3)_3$); ^{19}F NMR (188 MHz, $CDCl_3$) δ -30.4 (br s, COF); MS (EI) calcd for $C_{11}H_{20}NO_3F$, 233.1427; found 233.1427 (1%), 57.0703 (100%).

6-((6-Dimethylamino-2-naphthalenesulfonyl)amino)hexanoyl Fluoride (75).

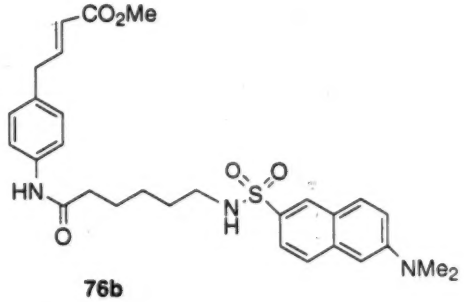


The general literature procedure was modified.[46] Compound **66** (100 mg, 0.28 mmol) was dissolved in CH_2Cl_2 (75 mL), cyanuric fluoride (35 mg, 0.26 mmol) and pyridine (25 mg, 0.30 mmol) were added. The solution was cooled to 0°C and stirred for 3 hours. The solution was then washed with H_2O (50 mL) and 1 M HCl (50 mL). The solution was dried ($MgSO_4$) and the solvent was removed. The product **75** (76 mg, 75%) was obtained as an oil: IR ($CHCl_3$ cast) 2926 (w), 1716 (m), 1620 (m), 1505 (m), 1383 (m), 1316 (m), 1148 (s) cm^{-1}; ^1H NMR (300 MHz, acetone-d6) δ 8.2 (d, J = 1.5Hz, 1H, aryl-C<u>H</u> ortho to SO_2), 7.8 (d, J = 9.2 Hz, 1H, aryl-C<u>H</u> meta to NMe_2), 7.8 (d, J = 8.8 Hz, 1H, aryl-C<u>H</u> ortho to SO_2), 7.6 (d of d, J = 8.8, 2.1 Hz, 1H, aryl-C<u>H</u> meta to SO_2), 7.3 (d of d, J = 9.2, 2.5 Hz, 1H, aryl-C<u>H</u> ortho to NMe_2), 7.00 (d, J = 2.1 Hz, 1H, aryl-C<u>H</u> ortho to NMe_2), 5.5 (t, J = 6.1 Hz, 1H, N<u>H</u>), 3.0 (s, 6H, N($C\underline{H}_3$)$_2$), 2.8 (t, J = 6.8 Hz, 2H, $C\underline{H}_2N$), 2.4 (t, 8.3 Hz, 2H, $C\underline{H}_2CO$), 1.5 (m, 4H, $CH_2C\underline{H}_2CH_2$), 1.4 (m, 2H, $CH_2C\underline{H}_2CH_2$); ^{13}C NMR (75 MHz, CD_3OD) δ 152.0 (<u>C</u>OF), 133.6, 131.0, 129.4, 128.2, 126.1, 123.7, 118.3, 106.4 (aryl <u>C</u>), 43.1 ($\underline{C}H_2COF$), 40.6 ($N\underline{C}H_3$), 30.3, 27.2, 25.6 ($\underline{C}H_2$); MS (posFAB) $C_{18}H_{18}N_2O_3FS$, 361.1; found 363.8 (31.2%), 119 (100%).

Methyl 4-[4-[*N*-(6-((*N*-(6-Dimethylamino-2-sulfonoamidonapthyl)) amino) hexanoyl)amino] phenyl]-3-butenoate (76a) and Methyl 4-[4-[*N*-(6-((*N*-(6-Dimethylamino-2-sulfonoamidonapthyl)) amino)hexanoyl)amino] phenyl]-2-butenoate (76b).



76a

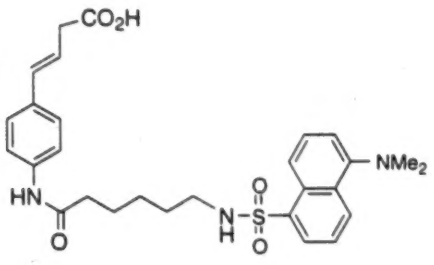


76b

The general coupling procedure was modified.[46] Amine **54** (100 mg, 0.50 mmol) and acid fluoride **75** (50 mg, 0.14 mmol) were dissolved in CH_2Cl_2 (100 mL) and 0.1M $NaHCO_3$ (50 mL), and the resulting biphasic solution was stirred for 8 hours. The solvent was removed and the residue was taken up in EtOAc (50 mL). This was washed with 1 N HCl (2x50 mL), saturated $NaHCO_3$ (2x50 mL), H_2O (50 mL) and saturated NaCl (50 mL). The solution was dried with $MgSO_4$ and the solvent was removed. The residue was further purified by flash chromatography (SiO_2, 20% EtOAc in hexane to 100% EtOAc, then 10% MeOH in EtOAc; R_f (**76 a** and **b**) 0.30 in 50% EtOAc). The product was obtained as a 5:1 mixture of **76 a** and **b** as a yellow solid (52 mg, 66%): HPLC (Novapak, RP C-18, monitored at 254 nm); solvent A: 0.1% TFA in water, solvent B 80/20/0.1 MeCN/$H_2$0/TFA; starting conditions 70/30 A/B changing to 40/60 A/B over 20 minutes, holding for 5 minutes, reverting to 70/30 A/B in 5 minutes and holding for 5 minutes; retention time of 13.3 minutes: IR ($CHCl_3$ cast) 3330 (w), 2927 (m), 1716 (m), 1683 (m), 1593 (m), 1411 (m), 1314 (m), 1206 (w), 1148 (s) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$, **76a**) δ 8.2 (s, 1H, aryl-C<u>H</u> ortho to SO_2), 7.8 (m, 1H, aryl-C<u>H</u> ortho to SO_2), 7.7 (m,

1H, aryl-C<u>H</u> meta to SO_2), 7.6 (m, 5H, aryl-C<u>H</u>) 7.2 (m, 2H, aryl-C<u>H</u>), 7.0 (d, J = 0.6 Hz, 1H, aryl-C<u>H</u> ortho to NMe_2), 6.4 (m, 2H, C<u>H</u>=CH), 5.0 (m, 1H, N<u>H</u>), 3.8 (s, 0.6H, $CO_2C\underline{H}_3$), 3.7 (s, 2.4H, $CO_2C\underline{H}_3$), 3.1 (s, 6H, $N(C\underline{H}_3)_2$), 3.5 (d, J = 7.6 Hz, 1H, $C\underline{H}_2CH{=}CH$), 3.2 (d, J = 7.6 Hz, 1H, $C\underline{H}_2CH{=}CH$), 2.9 (q, J = 6.8 Hz, 2H, $C\underline{H}_2N$), 2.3 (t, J = 7.2 Hz, 2H, $C\underline{H}_2CO$), 1.5 (m, 6H, $CH_2C\underline{H}_2CH_2$); ^{1}H NMR (300 MHz, $CDCl_3$, **76b**) δ 8.2 (s, 1H, aryl-C<u>H</u> ortho to SO_2), 7.8 (m, 1H, aryl-C<u>H</u> ortho to SO_2), 7.7 (m, 1H, aryl-C<u>H</u> meta to SO_2), 7.6 (m, 5H, aryl-C<u>H</u>) 7.2 (m, 3H, aryl-C<u>H</u>, C<u>H</u>=CH), 7.0 (d, J = 0.6 Hz, 1H, aryl-C<u>H</u> ortho to NMe_2), 5.9 (m, 1H, C<u>H</u>=CH), 5.0 (m, 1H, N<u>H</u>), 3.8 (s, 0.6H, $CO_2C\underline{H}_3$), 3.7 (s, 2.4H, $CO_2C\underline{H}_3$), 3.1 (s, 6H, $N(C\underline{H}_3)_2$), 3.5 (d, J = 7.6 Hz, 1H, $C\underline{H}_2CH{=}CH$), 3.2 (d, J = 7.6 Hz, 1H, $C\underline{H}_2CH{=}CH$), 2.9 (q, J = 6.8 Hz, 2H, $C\underline{H}_2N$), 2.3 (t, J = 7.2 Hz, 2H, $C\underline{H}_2CO$), 1.5 (m, 6H, $CH_2C\underline{H}_2CH_2$); MS (posFAB) $C_{29}H_{35}N_3O_5S$, 536.2219; found 537.4 (5%), 84.8 (100%).

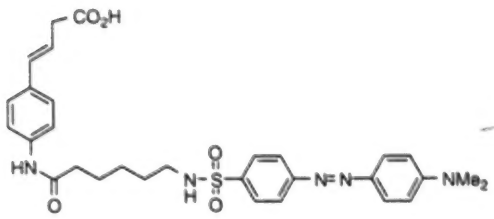
4-[4-[*N*-(6-((*N*-dansyl)amino)hexanoyl)amino]phenyl]-3-butenoic Acid (78).



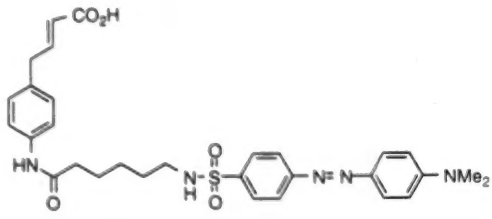
78

Compound **69** (30 mg, 0.056 mmol) and $LiOH \cdot H_2O$ (2.6 mg, 0.061 mmol) were dissolved in THF (5 mL) and H_2O (0.2 mL) and stirred for 18 hours. The solvent was removed and the residue was purified by reverse phase HPLC (Novapak, RP C-18, monitored at 254 nm); solvent A: 0.1% TFA in water, solvent B 80/20/0.1 MeCN/$H_2$0/TFA; starting conditions 70/30 A/B changing to 40/60 A/B over 20 minutes, holding for 5 minutes, reverting to 70/30 A/B in 5 minutes and holding for 5 minutes. The product **78** (6.4 mg, 22%) had a retention time of 15 minutes: IR (CH_3OH cast) 3300 (br), 3244 (w), 3174 (w), 3107 (w), 3042 (w), 2936 (m), 2859 (w), 1713 (m), 1666 (m), 1595 (m), 1529 (s), 1514 (s), 1462 (w), 1411 (m), 1316 (m), 1142 (s), 1094 (m), 791 (m) cm^{-1}; ^1H NMR (200 MHz, CD_3OD) δ 8.7 (m, 1H, aryl-C<u>H</u> ortho to SO_2), 8.5 (d, J = 8.6 Hz, 1H, aryl-C<u>H</u> para to NMe_2), 8.3 (d, J = 7.3 Hz, 1H, aryl-C<u>H</u> para to SO_2), 7.8 (m, 3H, aryl-C<u>H</u> meta to SO_2, ortho and meta to NMe_2), 7.4 (d, J = 8.5 Hz, 2H, aryl-C<u>H</u> ortho to NH), 7.3 (d, J = 8.5 Hz, 2H, aryl-C<u>H</u> meta to NH), 6.4 (d, J = 15.9 Hz, 1H, C<u>H</u>=CHCH$_2$), 6.2 (d of t, J = 15.9, 6.9 Hz, 1H, CH=C<u>H</u>CH$_2$), 3.2 (m, 8H, N(C<u>H</u>$_3$)$_2$, CH=CHC<u>H</u>$_2$), 2.9 (t, J = 6.5 Hz, 2H, C<u>H</u>$_2$N), 2.2 (t, J = 4.1 Hz, 2H, C<u>H</u>$_2$CO), 1.5-1.2 (m, 6H, C<u>H</u>$_2$C<u>H</u>$_2$C<u>H</u>$_2$); MS (EI) calcd for $C_{28}H_{33}N_3O_5$, 523.2141; found 523.2141 (17.32%), 171.1043 (100%).

4-[4-[*N*-(6-((*N*-Dabsyl)amino)hexanoyl)amino]phenyl]-3-butenoic Acid (79a) and 4-[4-[*N*-(6-((*N*-Dabsyl)amino)hexanoyl)amino]phenyl]-2-butenoic Acid (79b).



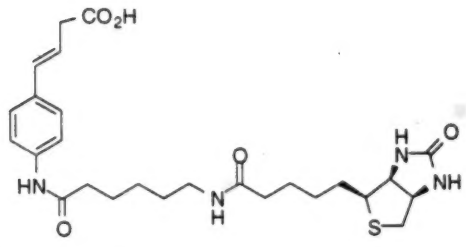
79a



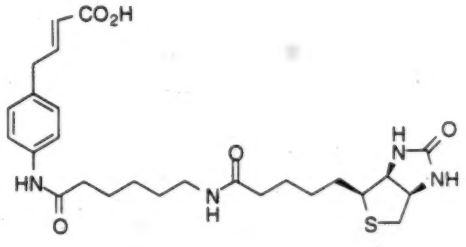
79b

Compound **71** (20 mg, 0.03 mmol) and $LiOH{\bullet}H_2O$ (2.5 mg, 0.06 mmol) were dissolved in THF (5 mL) and H_2O (0.2 mL) and stirred for 48 hours. The solvent was removed and the residue was purified by reverse phase HPLC (Novapak, RP C-18, monitored at 254 nm); solvent A: 0.1% TFA in water, solvent B 80/20/0.1 MeCN/$H_2$0/TFA; starting conditions 70/30 A/B changing to 40/60 A/B over 20 minutes, holding for 5 minutes, reverting to 70/30 A/B in 5 minutes and holding for 5 minutes. The product, a 1:1 ratio of **79a** and **b** (4 mg, 8%) had a retention time of 16.5 minutes: IR (μscope) 3315 (m), 2943 (m), 2872 (m), 1709 (s), 1425 (m), 1325 (s), 1255 (m), 1161 (s), 1087 (m), 845 (m) cm^{-1}; ^1H NMR (200 MHz, CD_3OD, **79a**) δ 7.9 (m, 5H, aryl-C<u>H</u>), 7.3 (m, 4H, aryl-C<u>H</u>), 7.0 (m, 4H, aryl-C<u>H</u>, N<u>H</u>), 6.3 (m, 2H, C<u>H</u>=CH), 3.5 (m, 2H, C<u>H</u>$_2$CH=), 3.2 (s, 6H, N(C<u>H</u>$_3$)$_2$), 2.9 (t, J = 7.0 Hz, 2H, C<u>H</u>$_2$NH), 2.4 (t, J = 6.3 Hz, 2H, C<u>H</u>$_2$CO), 1.6-1.4 (m, 6H, C<u>H</u>$_2$C<u>H</u>$_2$C<u>H</u>$_2$); ^1H NMR (200 MHz, CD_3OD, **79b**) δ 7.9 (m, 5H, aryl-C<u>H</u>), 7.3 (m, 4H, aryl-C<u>H</u>), 7.0 (m, 5H, aryl-C<u>H</u>, N<u>H</u>, C<u>H</u>=CH), 5.7 (m, 1H, C<u>H</u>=CH), 3.5 (m, 2H, C<u>H</u>$_2$CH=), 3.2 (s, 6H, N(C<u>H</u>$_3$)$_2$), 2.9 (t, J = 7.0 Hz, 2H, C<u>H</u>$_2$NH), 2.4 (t, J = 6.3 Hz, 2H, C<u>H</u>$_2$CO), 1.6-1.4 (m, 6H, C<u>H</u>$_2$C<u>H</u>$_2$C<u>H</u>$_2$); MS (posFAB) $C_{30}H_{35}N_5O_5S$, 577.2; found 578.5 (0.2%), 591.8 (0.6%, M+Na^+), 118.9 (100%).

4-[4-[*N*-(6-((*N*-Biotin)amino)hexanoyl)amino]phenyl]-3-butenoic Acid (80a) and 4-[4-[*N*-(6-((*N*-Biotin)amino)hexanoyl)amino]phenyl]-2-butenoic Acid (80b).



80a

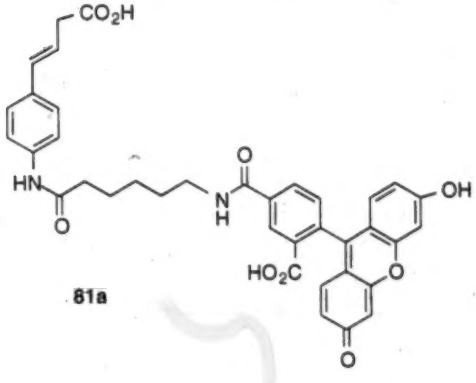


80b

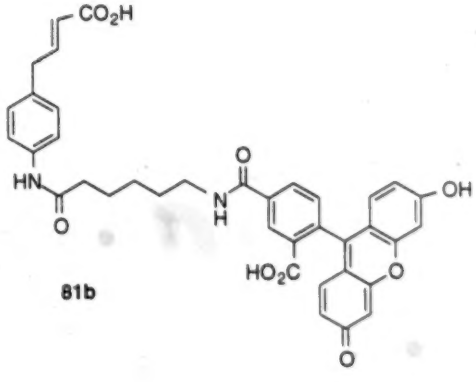
Compound **72** (50 mg, 0.01 mmol) and $LiOH{\bullet}H_2O$ (5 mg, 0.10 mmol) were dissolved in THF (5 mL) and H_2O (0.2 mL) and stirred for 72 hours. The solvent was removed and the residue was purified by reverse phase HPLC (Novapak, RP C-18, monitored at 254 nm); solvent A: 0.1% TFA in H_2O, solvent B 80/20/0.1 MeCN/$H_2$0/TFA; starting conditions 70/30 A/B changing to 40/60 A/B over 20 minutes, holding for 5 minutes, reverting to 70/30 A/B in 5 minutes and holding for 5 minutes. The product, a 11:1 ratio of **80a** and **b** (4 mg, 8%), had a retention time of 7 minutes: IR (μscope) 3426 (m, br), 3288 (s, br), 2931 (m), 2861 (m), 1697 (s), 1636 (s), 1607 (s), 1540 (m), 1465 (m), 1315 (m), 1268 (m), 1207 (m), 1162 (m), 965 (m) cm^{-1}; ^1H NMR (360 MHz, D_2O, **80a**) δ 7.5 (d, *J* = 8.4 Hz, 2H, aryl-C<u>H</u> ortho to NH), 7.4 (d, *J* = 8.4 Hz, 2H, aryl-C<u>H</u> meta to NH), 6.5, (d, *J* = 15.8 Hz, 1H, C<u>H</u>=CHCH$_2$), 6.4 (d of t, *J* = 15.8, 6.8 Hz, 1H, CH=C<u>H</u>CH$_2$), 4.5 (m, 1H, SCH$_2$C<u>H</u>), 4.3 (m, 1H, SCHRC<u>H</u>), 3.2 (m, 2H, CH=CHC<u>H$_2$</u>), 3.2 (m, 2H, C<u>H$_2$</u>N), 2.9 (d of d, *J* = 13.0, 4.9 Hz, 1H, SC<u>H</u>HCH), 2.7 (d, *J* = 12.8, 1H, SCH<u>H</u>CH), 2.4 (t, *J* = 6.8 Hz, 2H, C<u>H$_2$</u>CONH aryl), 2.2 (t, *J* = 7.2 Hz, C<u>H$_2$</u>CONHbiotin), 1.8-1.2 (m, 12H, CH$_2$C<u>H$_2$</u>CH$_2$); ^{13}C NMR (125 MHz, CD_3OD, HMQC, HMBC) δ 183.6 (<u>C</u>O$_2$H), 175.8 (NH<u>C</u>OCH$_2$biotin), 174.1 (arylNH<u>C</u>OCH$_2$), 166 (NH<u>C</u>ONH), 139.4 (<u>C</u>H=CHCH$_2$), 139.3 (ipso <u>C</u> amide), 135.7 (<u>C</u> ortho to vinyl), 134.2 (<u>C</u> ipso to vinyl), 133.9 (CH=<u>C</u>HCH$_2$), 130.2 (<u>C</u> ortho to amide), 63.4

(CHCHRS), 61.6 ($\underline{C}HCH_2S$), 56.9 ($\underline{C}HRS$), 52.3 ($CO_2\underline{C}H3$), 42.1 ($CH{=}CH\underline{C}H_2$), 41.0 ($\underline{C}H_2S$), 40.1 ($\underline{C}H_2NH$), 37.8 (arylNHCO$\underline{C}H_2$), 36.8 (NHCO$\underline{C}H_2$biotin), 30.1 (SCHR$\underline{C}H_2$), 29.7 (alkylNHCH$_2\underline{C}H_2$), 29.4 (biotinCOCH$_2\underline{C}H_2$), 27.5 (alkylNHCH$_2$ CH$_2\underline{C}H_2$), 26.9 (SCHRCH$_2\underline{C}H_2$), 26.4 (arylNHCOCH$_2\underline{C}H_2$); MS (posFAB) $C_{26}H_{35}N_4O_5S$, 515.6; found 517.5 (2.6%), 119.0 (100%).

4-[4-[*N*-(6-((*N*-(Fluorescein-5-carboxylic acid)) amino)hexanoyl) amino] phenyl]-3-butenoic Acid (81a) and 4-[4-[*N*-(6-((*N*-(Fluorescein-5-carboxylic acid)) amino)hexanoyl)amino]phenyl]-2-butenoic Acid (81b).



81a

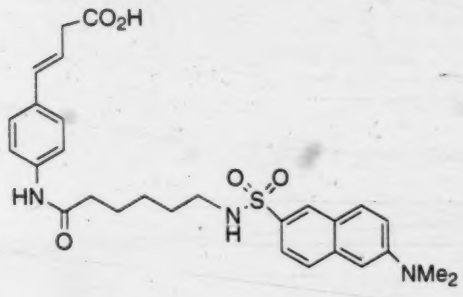


81b

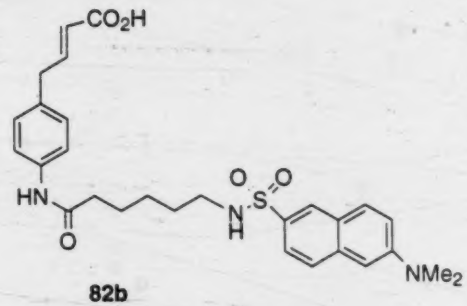
Compound **73 a** and **b** (20 mg, 0.03 mmol) and LiOH•H_2O (1.5 mg, 0.04 mmol) were dissolved in THF (10 mL) and H_2O (2 mL) and stirred for 72 hours. The solvent was removed and the residue was purified by reverse phase HPLC (Novapak, RP C-18, monitored at 254 nm); solvent A: 0.1% TFA in water, solvent B 80/20/0.1 MeCN/$H_2$0/TFA; starting conditions 70/30 A/B changing to 40/60 A/B over 20 minutes, holding for 5 minutes, reverting to 70/30 A/B in 5 minutes and holding for 5 minutes. The product, as a 20:1 ratio of **81 a** and **b** (2.2 mg, 13%) had a retention time of 7 minutes: IR (MeOH cast) 2939 (br, s), 2143 (m), 1694 (s), 1599 (s), 1532 (s), 1469 (m), 1233 (s), 1180 (m) cm^{-1}; ^1H NMR (300 MHz, CD_3OD, **81a**) δ 8.5 (d, J = 1.2 Hz, 1H, aryl-C$\underline{H}$

ortho to CO_2H), 8.1 (d of d, J = 8.6, 1.2 Hz, 1H, aryl-C<u>H</u> para to CO_2H), 7.5 (d, J = 9.4 Hz, 2H, aryl-C<u>H</u> ortho to NHCO), 7.3 (d, J = 9.4 Hz, 2H, aryl-C<u>H</u> para to NHCO), 7.2 (d, J = 8.6 Hz, 1H, aryl-C<u>H</u> meta to CO_2H), 6.8 (s, 2H, aryl-C<u>H</u> ortho to OH, vinyl <u>H</u> α to ketone), 6.7-6.6 (m, 4H, aryl-C<u>H</u> ortho and meta to hydroxyl, vinyl <u>H</u> α and β to ketone), 6.4 (d, J = 17.3 Hz, 1H, C<u>H</u>=CHCH$_2$), 6.2 (d of d, J = 17.3, 7.3 Hz, 1H, CH=C<u>H</u>CH$_2$), 3.5 (t, J = 7.3 Hz, 2H, C<u>H</u>$_2$N), 3.2 (d of d, J = 8.6, 1.4, 2H, CH=CHC<u>H</u>$_2$), 2.4 (t, J = 7.3, 2H, C<u>H</u>$_2$CO), 1.8 (m, 4H, CH$_2$C<u>H</u>$_2$CH$_2$), 1.6 (m, 2H, CH$_2$C<u>H</u>$_2$CH$_2$); MS (posFAB) $C_{37}H_{32}N_2O_9$, 648; found 649 (MH^+, 25%), 118.9 (100%).

4-[4-[*N*-(6-((*N*-(6-Dimethylamino-2-sulfonoamidonapthyl)) amino) hexanoyl) amino] phenyl]-3-butenoic Acid (82a) and 4-[4-[*N*-(6-((*N*-(6-Dimethylamino-2-sulfonoamidonapthyl)) amino)hexanoyl)amino]phenyl]-2-butenoic Acid (82b).



82a

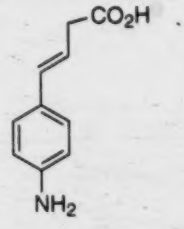


82b

Compound **76 a** and **b** (50 mg, 0.1 mmol) and $LiOH \cdot H_2O$ (5 mg, 0.10 mmol) were dissolved in THF (10 mL) and H_2O (2 mL) and stirred for 72 hours. The solvent was removed and the residue was purified by reverse phase HPLC (Novapak, RP C-18, monitored at 254 nm); solvent A: 0.1% TFA in H_2O, solvent B 80/20/0.1 MeCN/$H_2$0/TFA; starting conditions 70/30 A/B changing to 40/60 A/B over 20 minutes, holding for 5 minutes, reverting to 70/30 A/B in 5 minutes and holding for 5 minutes. The product, a 5:1 ratio of **82a** and **b** (7 mg, 13%) had a retention time of 12.5 minutes: IR ($CHCl_3$ cast) 3250 (br, m), 2924 (m), 1697 (m), 1598 (m), 1433 (m), 1314 (m), 1196 (w), 1148 (s) cm^{-1}; ^1H NMR (400 MHz, CD_3OD, **82a**) δ 8.2 (d, J = 1.7 Hz, 1H, aryl-C<u>H</u> ortho to SO_2), 7.8 (d, J = 9.1 Hz, 1H, aryl-C<u>H</u> ortho to SO_2), 7.7 (d, J = 8.8 Hz, 1H, aryl-C<u>H</u> meta to SO_2), 7.6 (m, 5H, aryl-C<u>H</u>) 7.2 (m, 2H, aryl-C<u>H</u>), 7.0 (d, J = 0.6 Hz, 1H, aryl-C<u>H</u> ortho to NMe_2), 6.4 (m, 2H, C<u>H</u>=CH), 3.1 (s, 6H, N(C<u>H</u>$_3$)$_2$), 2.8 (m, 4H, C<u>H</u>$_2$CH=CH, C<u>H</u>$_2$N), 2.3 (t, J = 7.2 Hz, 2H, C<u>H</u>$_2$CO), 1.5 (m, 6H, CH$_2$C<u>H</u>$_2$CH$_2$); ^1H NMR (400 MHz, CD_3OD, **82b**) δ 8.2 (d, J = 1.7 Hz, 1H, aryl-C<u>H</u> ortho to SO_2), 7.8 (d, J = 9.1 Hz, 1H, aryl-C<u>H</u> ortho to SO_2), 7.7 (d, J = 8.8 Hz, 1H, aryl-C<u>H</u> meta to SO_2), 7.6 (m, 5H, aryl-C<u>H</u>) 7.2 (m, 2H, aryl-C<u>H</u>, C<u>H</u>=CH), 7.0 (d, J = 0.6 Hz, 1H, aryl-C<u>H</u>

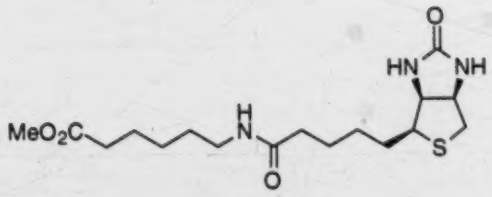
ortho to NMe_2), 5.9 (m, 1H, C<u>H</u>=CH), 3.1 (s, 6H, N(C<u>H</u>$_3$)$_2$), 2.8 (m, 4H, C<u>H</u>$_2$CH=CH, C<u>H</u>$_2$N), 2.3 (t, J = 7.2 Hz, 2H, C<u>H</u>$_2$CO), 1.5 (m, 6H, CH_2C<u>H</u>$_2CH_2$); MS (posFAB) $C_{28}H_{32}N_3O_5S$, 522.2; found 523 (5.1%), (118.1, 100%).

4-(4-Aminophenyl)-3-butenoic Acid (84).



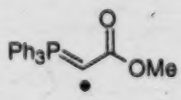
The literature procedure was modified.[47] The ester **54** (20 mg, 0.1 mmol) was dissolved in H_2O (0.5 mL) and THF (3 mL) and the solution was stirred for 16 hours. The solution was acidified to pH 2 with 1M HCl and extracted into EtOAc (3x10 mL). The solution was dried ($MgSO_4$) and the solvent was removed. The residue was purified by flash chromatography (SiO_2, EtOAc, then 5% MeOH in EtOAc, R_f 0.2 in EtOAc) to give the product **84** as a clear oil (5 mg, 28%): IR (CH_2Cl_2 cast) 3383 (s br), 2850 (m), 2579 (w), 1733 (s), 1607 (s), 1574 (m), 1511 (s), 1371 (m), 1204 (m), 1173 (m), 1084 (m), 968 (m) cm^{-1}; ^1H NMR (200 MHz, CD_3OD) δ 7.1 (d J = 8 Hz, 2H, aryl-C<u>H</u> ortho to alkene), 6.6 (d, J = 8 Hz, 2H, aryl-C<u>H</u> meta to alkene), 6.4 (d, J = 15.9 Hz, 1H, C<u>H</u>=CHCH$_2$), 6.0 (d of t, J = 15.9, 7.1 Hz, 1H, CH=C<u>H</u>CH$_2$), 3.2 (d of d, J = 7.1, 1.3 Hz, 2H, C<u>H</u>$_2$); MS (posFAB) $C_{10}H_{11}NO_2$, 177.1; found 200.1 (80.4%, M+Na$^+$), 177.0 (45%), 84.9 (100%).

Methyl 6-(*N*-Biotinylamino)hexanoate (86).



The general esterification procedure was modified.[52] 6-(*N*-Biotinylamino)hexanoic acid (10 mg, 0.027 mmol) was dissolved in acetonitrile (100 ml) and a solution of diazomethane in ether was added until a yellow colour persisted. The mixture was stirred for 3 hours and argon gas was bubbled through the solution for 30 minutes to remove any unreacted diazomethane. The solvent was removed to give the product **86** (93 mg, quantitative): IR (KBr disk) 3409 (br), 3300 (m), 2928 (m), 1738 (m), 1704 (s), 1641 (s), 1461 (m), 1264 (m), 1166 (m) cm^{-1}; ^1H NMR (300 MHz, CD_3OD) δ 4.5 (m, 1H, SCH_2CH), 4.3 (m, 1H, SCHRCH), 3.7 (s, 3H, CO_2CH_3) 3.2 (t, J = 6.7 Hz, 2H, CH_2N), 2.9 (d of d, J = 12.8, 4.8 Hz, 1H, SCHH'CH), 2.7 (d, J = 12.8, 1H, SCHH'CH), 2.4 (t, J = 7.2 Hz, 2H, CH_2CONH aryl), 2.2 (t, J = 7.3 Hz, 2H, CH_2CONHbiotin), 1.8-1.2 (m, 12H, $CH_2CH_2CH_2$); ^{13}C NMR (75 MHz, CD_3OD) δ 176.0, 62.0, 61.6, 57.0, 51.9, 41.0, 40.1, 36.8, 34.7, 29.8, 29.5, 27.5, 26.9, 25.7; MS (EI) calcd for $C_{17}H_{29}N_3O_4S$, 371.1879; found 371.1883 (1.4%), 114.0921 (100%).

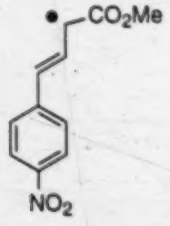
(Carboxymethylmethylene)-2-^{13}C-triphenylphosphorane (88).[75]



Methyl-2-bromo-2-^{13}C-acetate (0.80 g, 5.2 mmol) and triphenylphosphine (1.36 g, 5.2 mmol) were dissolved in benzene (30 mL) and the solution was heated to reflux and stirred

for 24 hours. The mixture was then filtered and the crystalline product was washed with toluene to give the intermediate phosphonium salt (1.0 g, 46%). The phosphonium salt (0.70 g, 1.7 mmol) was dissolved in dioxane/H_2O (30 mL) and sodium hydroxide (67 mg, 1.7 mmol, dissolved in H_2O (10 mL) was added. The solution was stirred for 1 hour and filtered to give an initial crop of **88**. The aqueous layer was extracted with EtOAc (3x50 mL), the organic phase was dried and the solvent was removed to give the remaining product. The solid was combined to give **88** (0.35 g, 59%): IR ($CHCl_3$ cast) 3055 (m), 1618 (m), 1589 (m), 1482 (m), 1436 (s), 1119 (s), 720 (s) cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) 7.5 (m, 15H, aryl-C<u>H</u>), 3.5 (s, 3H, $CO_2C\underline{H}_3$), 2.9 (s, 0.5H, $C\underline{H}CO_2CH_3$), 2.1 (s, 0.5H, $C\underline{H}CO_2CH_3$); MS (EI) calcd for $^{13}C^{12}C_{20}H_{19}O_2P$, 335.1122; found 335.1153 (4.8%), 277.0800 (100%).

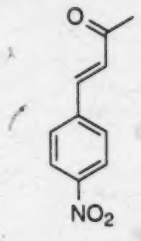
Methyl [2-^{13}C] 4-(4-Nitrophenyl)-3-butenoate (89).



The general procedure was modified.[36] (4-Nitrophenyl)acetaldehyde (**49**, 300 mg, 1.7 mmol) was dissolved in THF (30 mL) and Ph_3PCHCO_2Me (**88**) (0.30 g, 0.90 mmol) was added, and the solution was stirred for 16 hours. The solvent was removed and the residue was purified by column chromatography (Florisil, 10% EtOAc in hexane to 100% EtOAc, R_f 0.8 in 100% EtOAc) to yield the product **89** (150 mg, 75%); IR ($CHCl_3$ cast) 2952 (m), 1735 (m), 1597 (m), 1518 (s), 1345 (s), 1204 (s), 1166 (m), 857 (m) cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) 7.8 (d, J = 8.8 Hz, 2H, aryl-C<u>H</u> ortho to NO_2), 6.7 (d, J = 8.8 Hz, 2H, aryl-C<u>H</u> meta to NO_2), 6.1 (m, 2H, C<u>H</u>=C<u>H</u>), 3.3 (s, 3H, CH_3), 2.8 (m, 2H, $C\underline{H}_2$-

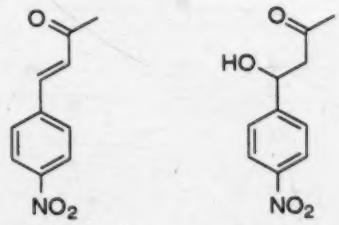
CH);MS (EI) calcd for $^{13}C^{12}C_{10}H_{11}NO_4$, 222.0688; found 222.0722 (54.7%), 117.0658 (100%).

4-(4-Nitrophenyl)-3-buten-2-one (91) From Dehydration of 92.[69]



4-(4-Nitrophenyl)-3-hydroxy-butan-2-one (**92**) (260 mg, 1.2 mmol) was dissolved in benzene (50 mL) and para-toluenesulfonic acid (20 mg, 0.16 mmol) was added. The flask was fitted with a Soxhlet extractor with a thimble containing alternating layers of CaH_2 and glass wool. The solution was heated to reflux and stirred for 24 hours . After cooling to room temperature, the mixture was washed with H_2O (15 mL), saturated $NaHCO_3$ (15 mL) and H_2O (15 mL). The organic phase was dried with Na_2SO_4, and the solvent was removed. Purification by flash chromatography (SiO_2, EtOAc/hexane, 25/75 to 100/0; R_f 0.80 (EtOAc)) gave **91** (130 mg, 90%) as a yellow oil: IR ($CHCl_3$ cast) 3002 (w), 1712 (s), 1673 (s), 1597 (m), 1517 (s), 1494 (m), 1346 (s), 1256 (m), 1177 (m), 858 (m) cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 8.3 (d, J = 8 Hz, 2H, C<u>H</u> aryl, ortho to NO_2), 7.7 (d, J = 8 Hz, 2H, C<u>H</u> aryl, meta to NO_2), 7.6 (d, J = 16 Hz, 1H, C<u>H</u>= para to NO_2), 6.8 (d, J = 16 Hz, 1H, C<u>H</u>=), 2.4 (s, 3H, C<u>H</u>$_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 206.3 (<u>C</u>O ketone), 146.8, 145.4, 140.7, 130.4, 128.8, 124.2 (aryl, vinyl <u>C</u>), 50.5 (<u>C</u>H$_3$); MS (EI) calcd for $C_{10}H_9NO_3$, 191.0582; found 191.0588 (25.62%), 176.0350 (100%).

4-(4-Nitrophenyl)-3-buten-2-one (91)[69] and 4-(4-nitrophenyl)-4-hydroxy-butan-2-one (92).[69]

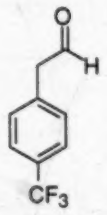


91 **92**

The literature procedure was modified.[36] 4-Nitrobenzaldehyde (3.0 g, 20 mmol) was dissolved in THF (40 mL) and acetone (3.48 g, 60 mmol) was added followed by aqueous KOH (370 µL, 15% KOH, 1.0 mmol). The mixture was stirred for 3 hours, the pH was adjusted to 6 with glacial acetic acid, and the solution was diluted with H_2O (200 mL) and EtOAc (300 mL). The organic extract was washed with a saturated sodium chloride solution (2x50 mL), filtered and extracted with EtOAc (3x150 mL). The solutions were dried with Na_2SO_4 and the solvent was removed. The crude product was purified by flash chromatography (SiO_2, EtOAc/ hexane, 25/75 to 100/0, then EtOAc/ MeOH, 90/10; R_f 0.80 (**91**) (EtOAc), R_f 0.50 (**92**) (EtOAc)). Compound **91** was further purified by flash chromatography (SiO_2, EtOAc/ hexane, 25/75 to 100/0). The alkene **91** (0.50 g, 13%) (see below for data) and the alcohol **92** (3.00 g, 72%) were obtained as yellow oils. The alcohol (**92**) was characterized as follows: IR ($CHCl_3$ cast) 3388 (br), 1727 (s), 1602 (m), 1518 (s), 1496 (m), 1453 (w), 1434 (w), 1345 (s), 1320 (m), 1320 (m), 1281 (m), 1184 (m), 748 (s), 698 (s) cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 8.2 (d, J = 7.8 Hz, 2H, aryl C<u>H</u> ortho to NO_2), 7.5 (d, J = 7.8 Hz, 2H, aryl C<u>H</u> meta to NO_2), 5.3 (d of t, J = 7.6, 5.1 Hz, 1H, C<u>H</u>OH), 3.9 (d, J =5.1 Hz, 1H, CHO<u>H</u>), 2.9 (d, J = 7.6 Hz, 2H, C<u>H</u>$_2$), 2.2 (s, 3H, C<u>H</u>$_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 208.4 (<u>C</u>O, ketone), 150.0, 147.3, 126.3,

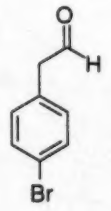
123.7 (aryl C), 68.9 (COH), 51.5 (CH_2), 30.7 (CH_3); MS (EI) calcd for $C_{10}H_{11}NO_4$, 209.0688; found 209.0686 (64.3%) 58.0452 (100%).

2-(4-(Trifluoromethyl)phenyl)acetaldehyde (94).



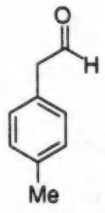
4-(Trifluoromethyl)styrene (Pfaltz & Bauer, 1.00 g, 5.7 mmol) was dissolved in CH_2Cl_2 (100 mL). This solution was added to $Pb(OAc)_4$ (3.00 g, 6.7 mmol) in trifluoroacetic acid (100 mL), and the mixture was stirred for 2 hours. The mixture was then washed with H_2O (50 mL) and saturated $NaHCO_3$ (50 mL). The organic extracts were dried with $MgSO_4$ and the solvent was removed to yield the product **94** as a yellow oil (0.50 g, 47%): IR ($CHCl_3$) 2937, 1789 (s), 1744 (w), 1620 (m), 1438 (w), 1420 (m), 1376 (w), 1327 (s), 1228 (m), 1164 (s), 1124 (s), 1068 (s), 841 (m) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 9.8 (t, J = 1.7 Hz, 1H, CHO), 7.6 (d, J = 8.3 Hz, 2H, aryl-CH ortho to CF_3), 7.4 (d, J = 8.3, 2H, aryl-CH meta to CF_3), 3.9 (d, J = 1.7 Hz, 2H, CH_2); MS (EI) calcd for $C_9H_7OF_3$, 188.0449; found 188.0447 (40.38%), 159.0419 (100%).

2-(4-Bromophenyl)acetaldehyde (95).[70]



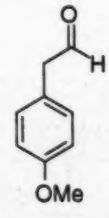
4-Bromostyrene (Pfaltz & Bauer, 1.00 g, 5.5 mmol) was dissolved in CH_2Cl_2 (100 mL). This solution was added to $Pb(OAc)_4$ (1.8 g, 0.46 mmol) in trifluoroacetic acid (100 mL) and the mixture was stirred for 2 hours. The mixture was washed with H_2O (50 mL) and saturated $NaHCO_3$ (50 mL). The organic extracts were dried with $MgSO_4$ and the solvent was removed to yield the product **95** as a yellow oil (0.65 g, 72%): IR ($CHCl_3$ cast) 2934 (w), 1813 (s), 1726 (m), 1594 (w), 1490 (m), 1437 (w), 1381 (m), 1230 (s), 1175 (s), 1159 (s), 1114 (s), 1073 (s), 1026 (m), 976 (m), 740 (m) cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) δ 9.8 (t, J = 2.1 Hz, 1H, C<u>H</u>O), 7.5 (d, J = 6.5 Hz, 2H, aryl-C<u>H</u> ortho to Br), 7.0 (d, J = 6.5 Hz, 2H, aryl-C<u>H</u> meta to Br), 3.7 (d, J = 2.1 Hz, 2H, C<u>H</u>$_2$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 198.7 (<u>C</u>HO), 132.0 (aryl-<u>C</u>H ortho to Br), 131.3 (aryl-<u>C</u>H meta to Br), 130.1 (aryl-<u>C</u>, CH_2 attached), 122.4 (aryl-<u>C</u>, Br attached), 38.6 (<u>C</u>H_2); MS calcd for $C_8H_7O^{79}Br$, 197.9660; $C_8H_7O^{81}Br$, 199.9660; found 199.9672 30.6%, 168.9655 (100%).

4-(Methylphenyl)-2-acetaldehyde (96).[35]



4-Methylstyrene (1.00 g, 8.5 mmol) was dissolved in CH_2Cl_2 (100 mL). This solution was added to $Pb(OAc)_4$ (2.2 g, 5.0 mmol) in trifluoroacetic acid (100 mL), and stirred for 2 hours. The mixture was then washed with H_2O (50 mL), and saturated $NaHCO_3$ (50 mL). The organic extracts were dried with $MgSO_4$ and the solvent was removed to yield the product **96** as a yellow oil (0.42 g, 63%): IR ($CHCl_3$ cast) 3018 (w), 2922 (m), 2860 (w), 1783 (m), 1513 (m), 1377 (m), 1222 (m), 1169 (m), 814 (m) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 9.7 (t, J = 3.5 Hz, 1H, C<u>H</u>O), 7.2 (d, J = 6.2 Hz, 2H, aryl-C<u>H</u> meta to CH_3), 7.1 (d, J = 6.2 Hz, 2H, aryl-C<u>H</u> ortho to CH_3), 2.8 (d, J = 3.5 Hz, 2H, C<u>H</u>$_2$); MS calcd for $C_9H_{10}O$, 134.0731; found 134.0732 (26.4%), 105.0705 (100%).

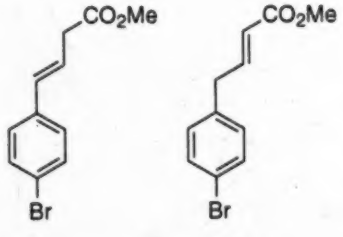
2-(4-Methoxyphenyl)acetaldehyde (97).



4-Methoxystyrene (1.00 g, 7.5 mmol) was dissolved in MeOH (10 mL). This solution was added to HgO (0.32 g, 1.5 mmol) and the solution was heated to reflux with stirring for 1 hour. The mixture was then washed with H_2O (50 mL). The organic extracts was

dried with $MgSO_4$ and the solvent was removed to yield the product **97** as a yellow oil (0.72g, 64%): IR (KBr disk) 2997 (w), 2834 (w), 1611 (s), 1583 (m), 1512 (s), 1462 (m), 1301 (m), 1247 (s), 1177 (m), 1033 (m), 829 (m) cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) δ 9.7 (t, *J* = 2.1 Hz, 1H, C<u>H</u>O), 7.2 (d, *J* = 9.0 Hz, 2H, aryl-C<u>H</u> meta to OCH_3), 6.9 (d, *J* = 9.0 Hz, 2H, aryl-C<u>H</u> ortho to OCH_3), 3.9 (s, 3H, $OC\underline{H}_3$), 3.6 (d, *J* = 2.1 Hz, 2H, $C\underline{H}_2$); MS calcd for $C_9H_{10}O_2$, 150.0681; found 150.0679 (27.4%), 121.0653 (100%).

Methyl 4-(4-Bromophenyl)-3-butenoate (99a) and Methyl 4-(4-Bromophenyl-2-butenoate (99b).

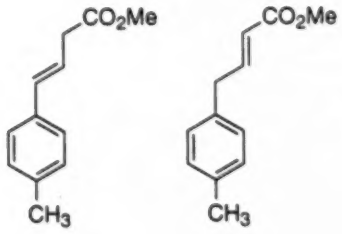


99a **99b**

The literature procedure was modified. (4-Bromophenyl)acetaldehyde (**95**) (500 mg, 2.7 mmol) was dissolved in THF (30 mL), Ph_3PCHCO_2Me (**51**) (1.5 g, 4.8 mmol) was added, and the solution was stirred for 16 hours. The solvent was removed and the residue was purified by column chromatography (Florisil, 10% EtOAc in hexane to 50% EtOAc, R_f 0.55 in 50% EtOAc) to yield the product (0.43 g, 89%) as a yellow oil as a 4:1 mixture of **99a** and **b** : IR ($CHCl_3$) 3024 (w), 2994 (w), 1783 (m), 1721 (s), 1656 (m), 1487 (m), 1435 (m), 1333 (m), 1274 (s), 1162 (s), 1011 (s), 822 (m), 796 (m) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$, **99a**) δ 7.4 (d, *J* = 8.8 Hz, 2H, aryl-C<u>H</u> ortho to Br), 7.0 (m, 3H, aryl-C<u>H</u> meta to Br, $CH_2CH{=}C\underline{H}$), 6.7 (d, *J* = 15.9 Hz, 1H, $C\underline{H}{=}CHCH_2$), 6.4 (d of t, *J* = 15.9, 7.0 Hz, $CH{=}C\underline{H}CH_2$), 3.7 (s, 3H, $CO_2C\underline{H}_3$), 3.2 (d, *J* = 7.0 Hz, 2H, $C\underline{H}_2CH{=}CH$); ^1H NMR (300 MHz, $CDCl_3$, **99b**) δ 7.4 (d, *J* = 8.8 Hz, 2H, aryl-C<u>H</u> ortho to Br), 7.0 (m,

3H, aryl-CH meta to Br, $CH_2CH{=}CH$), 5.8 (d of t, J = 15.8, 1.1 Hz, 1H, $CH_2CH{=}CH$), 3.7 (s, 3H, CO_2CH_3), 3.4 (d, J = 6.8 Hz, 2H, $CH_2CH{=}CH$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.7 (CO acid), 146.7, 136.5, 132.2, 131.7, 131.5, 130.5, 122.3, 120.6 (aryl, vinyl C), 51.5 (CH_3O), 37.7 (CH_2); MS calcd for $C_{11}H_{11}O_2{}^{79}Br$, 253.9942; $C_{11}H_{11}O_2{}^{81}Br$, 255.9942; found 253.9928 (40%), 149.0237 (100%).

Methyl 4-(4-Methylphenyl)-3-butenoate (100a) and Methyl 4-(4-Methyl phenyl)-2-butenoate (100b).[54]

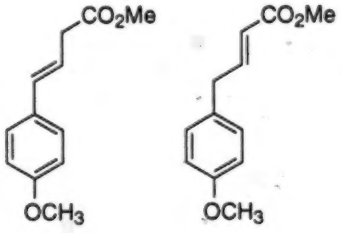


100a **100b**

The literature procedure was modified. 4-Methylphenyl acetaldehyde (**96**) (300 mg, 2.2 mmol) was dissolved in THF (30 mL), Ph_3PCHCO_2Me (**51**) (0.9 g, 3.6 mmol) was added, and the solution was stirred for 16 hours. The solvent was removed and the residue was purified by column chromatography (Florisil, 10% EtOAc in hexane to 50% EtOAc, R_f 0.40 in 50% EtOAc) to yield the product as a 1:1 mixture of **100a** and **100b** (150 mg, 36%): IR ($CHCl_3$ cast) 2951 (w), 1725 (s), 1671 (m), 1435 (m), 1304 (s), 1272 (s), 1167 (s), 1181 (m), 980 (m) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$, **100a**) δ 7.2 (m, 4H, aryl-CH), 6.5 (d, J = 15.9 Hz, 0.5H, $CH{=}CHCH_2$), 6.4 (d of t, J = 15.9, 6.8 Hz, 0.5H, $CH{=}CHCH_2$), 3.7 (s, 3H, OCH_3), 3.5 (d, J = 6.8 Hz, 2H, CH_2), 2.4 (s, 3H, CH_3); ^1H NMR (300 MHz, $CDCl_3$, **100b**) δ 7.2 (m, 5H, aryl-CH), 5.8 (d, J = 15.8 Hz, 1H, $CH_2CH{=}CH$), 3.7 (s, 3H, OCH_3), 3.5 (d, J = 6.8 Hz, 2H, CH_2), 2.4 (s, 3H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.0 (CO), 136.8, 129.6, 129.4, 129.0, 128.7, 120.8 (aryl

C, vinyl C), 53.5, 38.0, 29.7 (alkyl C); MS (posFAB) $C_{12}H_{14}O_2$, 190.1; found 191.1 (9%), 119.1 (100%).

Methyl 4-(4-Methoxyphenyl)-3-butenoate (101a) and Methyl 4-(4-Methoxy phenyl)-2-butenoate (101b).[54]

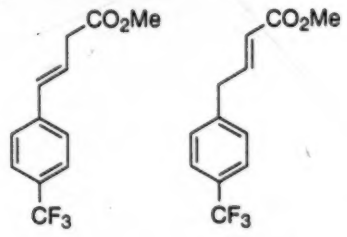


101a **101b**

The general literature procedure was modified.[23] 4-(Methoxyphenyl)acetaldehyde (**97**) (0.50 g, 3.3 mmol) was dissolved in THF (30 mL), Ph_3PCHCO_2Me (**51**) (1.50 g, 4.5 mmol) was added, and the solution was stirred for 16 hours. The solvent was removed and the residue was purified by column chromatography (Florisil, 10% EtOAc in hexane to 50% EtOAc, R_f 0.80 in 50% EtOAc) to yield the product as a 1:4 mixture of **101a** and **101b** (230 mg, 34%): IR ($CHCl_3$ cast) 3009 (w), 2954 (w), 2927 (w), 1721 (s), 1610 (m), 1511 (s), 1301 (m), 1247 (s), 1126 (s), 1033 (m), 827 (m) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$, **100a**) δ 7.1 (d, J = 8.8 Hz, 2H, aryl-CH meta to OCH_3), 6.8 (d, J = 8.8 Hz, 4H, aryl-CH ortho to OCH_3, CH=CH), 3.8 (s, 3H, CO_2CH_3), 3.7 (s, 3H, OCH_3), 3.4 (d, J = 6.3 Hz, 2H, CH_2CH=CH); ^1H NMR (300 MHz, $CDCl_3$, **100b**) δ 7.1 (d of t, J = 15.3, 6.3 Hz, 1H, CH_2CH=CH), 7.1 (d, J = 8.8 Hz, 2H, aryl-CH meta to OCH_3), 6.8 (d, J = 8.8 Hz, 2H, aryl-CH ortho to OCH_3), 5.8 (d of t, J = 15.3, 1.4 Hz, 1H, CH_2CH=CH), 3.8 (s, 3H, CO_2CH_3), 3.7 (s, 3H, OCH_3), 3.4 (d, J = 6.3 Hz, 2H, CH_2CH=CH); ^{13}C NMR (100 MHz, CD_3OD) δ 181.1 (CO), 158.2, 130.9, 130.0, 128.6, 127.4, 123.7, 114.3, 114.0 (aryl C, vinyl C), 55.4 (CH_2), 49.2 (CH_3 ester); MS (EI)

calcd for $C_{12}H_{14}O_3$, 206.0943; found 206.0939; Anal. Calcd for $C_{12}H_{14}O_3$; C, 69.89; H, 6.84. Found: C, 69.85; H, 7.02.

Methyl 4-(4-(Trifluoromethyl)phenyl)-3-butenoate (102a) and Methyl 4-(4-(Trifluoromethyl)phenyl-2-butenoate (102b).

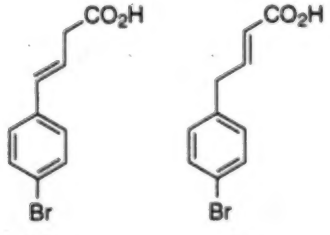


102a **102b**

The general literature procedure was modified.[23]: (4-(Trifluoromethyl)phenyl) acetaldehyde (**94**), (400 mg, 2.1 mmol) was dissolved in THF (30 mL), Ph_3PCHCO_2Me (**51**) (1.2 g, 3.6 mmol) was added, and the solution was stirred for 16 hours. The solvent was removed and the residue was purified by column chromatography (Florisil, 10% EtOAc in hexane to 50% EtOAc, R_f 0.40 in 50% EtOAc) to yield the product as a 7:1 mixture of **102a** and **102b** (150 mg, 30%): IR ($CHCl_3$ cast) 2953 (w), 1724 (s), 1658 (m), 1618 (m), 1437 (m), 1418 (m), 1326 (s), 1276 (m), 1209 (m), 1164 (s), 1124 (s), 1067 (s), 1019 (s), 818 (m) cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$, **102a**) δ 7.6 (d, J = 8.2 Hz, 2H, aryl-C<u>H</u> ortho to CF_3), 7.3 (d, J = 8.2, 2H, aryl-C<u>H</u> meta to CF_3), 7.1 (d of t, J = 15.9, 6.8 Hz, 1H, $CH_2C\underline{H}$=CH), 6.5 (d, J = 15.9 Hz, 1H, C<u>H</u>=$CHCH_2$), 6.4 (d of t, J = 15.9, 6.6 Hz, 1H, CH=C<u>H</u>CH_2), 3.7 (s, 3H, $CO_2C\underline{H}_3$), 3.2 (d, J = 6.6 Hz, 0.2H, CH=CHC<u>H</u>$_2$); ^1H NMR (360 MHz, $CDCl_3$, **102b**) δ 7.6 (d, J = 8.2 Hz, 2H, aryl-C<u>H</u> ortho to CF_3), 7.3 (d, J = 8.2, 2H, aryl-C<u>H</u> meta to CF_3), 7.1 (d of t, J = 15.9, 6.8 Hz, 1H, $CH_2C\underline{H}$=CH), 5.8 (d, J = 15.9 Hz, 1H, CH_2CH=C<u>H</u>), 3.7 (s, 3H, $CO_2C\underline{H}_3$), 3.6 (d, J = 6.8 Hz, 2H, C<u>H</u>$_2$CH=CH); MS (EI) calcd for $C_{12}H_{11}O_2F_3$, 244.0711; found

244.0712 (100%); Anal. Calcd for $C_{12}H_{11}O_2F_3$: C, 59.02; H, 4.54. Found: C, 58.74; H, 4.51.

4-(4-Bromophenyl)-3-butenoic Acid (103a) and 4-(4-Bromophenyl-2-butenoic Acid (103b).[70]

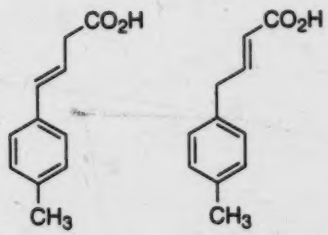


103a **103b**

Compound mixture **99ab** (200 mg, 0.78 mmol) and $LiOH•H_2O$ (30 mg, 0.78 mmol) were dissolved in THF (10 mL) and H_2O (3 mL) and stirred for 24 hours. The solvents were removed and the residue was purified by reverse phase HPLC (Novapak, RP C-18, monitored at 254 nm); solvent A: 0.1% TFA in H_2O, solvent B 80/20/0.1 MeCN/$H_2$0/TFA; starting conditions 70/30 A/B changing to 40/60 A/B over 20 minutes, holding for 5 minutes, reverting to 70/30 A/B in 5 minutes and holding for 5 minutes. The product, a 1:1 mixture of **103a** and **103b** (25 mg, 13%), had a retention time of 19 minutes: IR (μscope) 3300 (m, br), 1584 (s), 1565 (m), 1487 (m), 1421 (m), 1304 (m), 1072 (m), 966 (w) cm^{-1}; ^1H NMR (300 MHz, CD_3OD, **103a**) δ 7.5 (d, J = 7.5 Hz, 2H, aryl-C<u>H</u> ortho to Br), 7.3 (d, J = 7.5 Hz, 2H, aryl-C<u>H</u> meta to Br), 6.4 (d, J = 16.0 Hz, 1H, C<u>H</u>=CHCH$_2$), 6.3 (d of t, J = 16.0, 6.8 Hz, 1H, CH=C<u>H</u>CH$_2$), 3.1 (d, J = 6.8 Hz, 2H, C<u>H</u>$_2$); ^1H NMR (300 MHz, CD_3OD, **103b**) δ 7.5 (d, J = 7.5 Hz, 2H, aryl-C<u>H</u> ortho to Br), 7.3 (d, J = 7.5 Hz, 2H, aryl-C<u>H</u> meta to Br), 6.9 (d, J = 15.9 Hz, 1H, CH$_2$CH=C<u>H</u>), 5.8 (d of t, J = 15.9, 6.8 Hz, 1H, CH$_2$C<u>H</u>=CH), 3.2 (d of d, J = 6.8, 0.8 Hz, 2H, C<u>H</u>$_2$); ^{13}C NMR (100 MHz, CD_3OD) δ 179.8 (<u>C</u>O, acid), 138.4, 132.5, 130.9,

128.8, 128.5, 121.3 (aryl, vinyl C), 43.4 ($\underline{C}H_2$); MS (EI) calcd for $C_{10}H_9O_2{}^{81}Br$, 241.9765; $C_{10}H_9O_2{}^{79}Br$, 239.9765; found 241.9775 (3%), 198.9756 (100%).

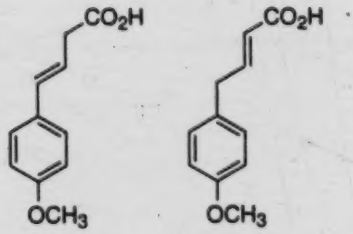
4-(4-Methylphenyl)-3-butenoic Acid (104a) and 4-(4-Methylphenyl-2-butenoic acid (104b).[71]



104a **104b**

Compound mixture **100ab** (100 mg, 0.53 mmol) and $LiOH{\bullet}H_2O$ (30 mg, 0.78 mmol) were dissolved in THF (10 mL) and H_2O (3 mL) and stirred for 24 hours. The solvents were removed and the residue was purified by reverse phase HPLC (Novapak, RP C-18, monitored at 254 nm); solvent A: 0.1% TFA in H_2O, solvent B 80/20/0.1 MeCN/$H_2$0/TFA; starting conditions 70/30 A/B changing to 40/60 A/B over 20 minutes, holding for 5 minutes, reverting to 70/30 A/B in 5 minutes and holding for 5 minutes. The product, a 1:1 mixture of **104a** and **104b** (15 mg, 16%) had a retention time of 16.5 minutes: IR (KBr disk) 3441 (s, br), 3024 (w), 2920 (w), 2858 (w), 1735 (s), 1655 (s), 1573 (s), 1429 (s), 1319 (w), 1169 (w), 1032 (w), 771 (m), 559 (m) cm^{-1}; ^1H NMR (300, MHz, CD_3OD, **104a**) δ 7.3 (d, J = 8.4 Hz, 2H, aryl-C$\underline{H}$ ortho to alkene), 7.1 (d, J = 8.4 Hz, 2H, aryl-C$\underline{H}$ ortho to CH_3), 6.5 (d, J = 15.9, 1H, C$\underline{H}$=CHCH$_2$), 6.3 (d of t, J = 15.9, 7.1, 1H, CH=C$\underline{H}$CH$_2$), 3.2 (d of d, J = 7.1, 1.1 Hz, 2H, $\underline{C}H_2$), 2.3 (s, 3H, $\underline{C}H_3$); ^{13}C NMR (100 MHz, CD_3OD) δ 177 ($\underline{C}$O acid), 138.3, 135.7, 134.2, 130.5, 130.2, 127.2, 126, 122.2 (aryl, vinyl $\underline{C}$), 38 ($\underline{C}H_2$), 22 ($\underline{C}H_3$); MS (EI) calcd for $C_{11}H_{12}O_2$, 176.0837; found 176.0832 (3.6%), 121.0655 (100%).

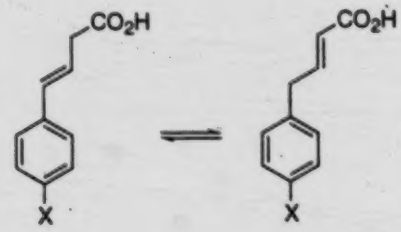
4-(4-Methoxyphenyl)-3-butenoic Acid (105a) and 4-(4-methoxy phenyl)-2-butenoic Acid (105b).[71]



105a **105b**

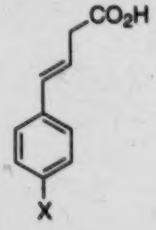
Compound mixture **101ab** (200 mg, 0.97 mmol) and $LiOH \cdot H_2O$ (50 mg, 1.2 mmol) were dissolved in THF (15 mL) and H_2O (5 mL) and stirred for 24 hours. The solvent was removed and the residue was purified by reverse phase HPLC (Novapak, RP C-18, monitored at 254 nm); solvent A: 0.1% TFA in H_2O, solvent B 80/20/0.1 MeCN/$H_2$0/TFA; starting conditions 70/30 A/B changing to 40/60 A/B over 20 minutes, holding for 5 minutes, reverting to 70/30 A/B in 5 minutes and holding for 5 minutes. The product, a 1:4 mixture of **105a** and **105b** (21 mg, 11%) had a retention time of 14.5 minutes: IR ($CHCl_3$ cast) 3444 (br), 2922 (m), 1653 (m), 1569 (m), 1419 (m), 1300 (m), 1246 (m), 1173 (m), 1030 (m), 772 (m) cm^{-1}; ^1H NMR (300 MHz, CD_3OD, **105a**) δ 7.3 (d, J = 8.8 Hz, 2H, aryl-C<u>H</u> meta to OMe), 6.8 (d, J = 8.8 Hz, 2H, aryl-C<u>H</u> ortho to OMe), 6.4 (d, J = 15.9 Hz, 1H, C<u>H</u>=CHCH$_2$), 6.3 (d of t, J = 15.9, 6.1 Hz, 1H, CH=C<u>H</u>CH$_2$), 3.8 (s, 3H, OC<u>H</u>$_3$), 3.1 (d, J = 6.1 Hz, 2H, C<u>H</u>$_2$); ^1H NMR (300 MHz, CD_3OD, **105b**) δ 7.3 (d, J = 8.8 Hz, 2H, aryl-C<u>H</u> meta to OMe), 6.9 (d of t, J = 16.0, 6.9 Hz, 1H, CH$_2$C<u>H</u>=CH), 6.8 (d, J = 8.8 Hz, 2H, aryl-C<u>H</u> ortho to OMe), 5.8 (d, J = 16.0 Hz, 1H, CH$_2$CH=C<u>H</u>), 3.8 (s, 3H, OC<u>H</u>$_3$), 3.0 (d, J = 6.9 Hz, 1.6H, C<u>H</u>$_2$); ^{13}C NMR (100 MHz, CD_3OD) δ 167.0 (<u>C</u>O), 132.8, 131.0, 128.0, 115.7, 115.4, 115.2, 114.8, 105.0 (aryl <u>C</u>, vinyl <u>C</u>), 55.8 (<u>C</u>H$_2$); MS calcd for $C_{11}H_{12}O_3$, 192.0786; found 192.0783 (5.4%), 149.0239 (100%).

NMR Study of Double Bond Isomerization of 4-Phenylbutenoic acids (X=H, Br, Me, OMe).



The appropriate phenylbutenoic acid (10 mg, 0.06 mmol to 0.04 mmol) was dissolved in a solution of $LiOH \cdot H_2O$ (3.5 mg, 0.10 mmol) in D_2O (0.5 mL). The isomerization was monitored at regular intervals by NMR spectrometry for a period of 77 hours. Isomerization occurred slowly to give predominantly the β,γ-unsaturated acid. However, a small amount (<5%) of the α,β-unsaturated isomer remained. The acid was then desalted by applying the aqueous solution to a cation exchange Sep-pak Plus, washing with water and then eluting with acetonitrile to give the free acid for the crystallization study below.

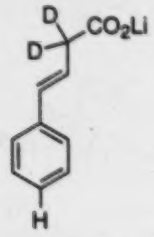
Recrystallization of 4-Phenyl-3-butenoic Acids (X=H, Br, Me, OMe).



The appropriate phenylbutenoic acid (10 mg, 0.06 mmol to 0.04 mmol) obtained from the NMR study above, was dissolved in a minimum amount of hot EtOAc. The solution was cooled to room temperature and hexane was added until the solution became cloudy. The mixture was cooled to 4°C and left to crystallize for 48 hours. The solution was filtered to

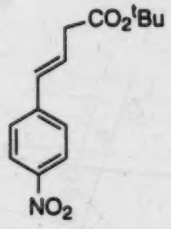
give the pure 4-phenyl)-3-butenoic acid. The chromatographic and spectral properties were nearly identical to the mixtures **103a,b**, **104a,b**, **105a,b** described above except in the ^1H NMR where the olefinic resonances and the methylene resonances of the minor isomer have disappeared.

[2,2-$^2H_2$]-4-Phenyl-3-butenoic Acid Lithium Salt (106).



4-Phenyl-3-butenoic acid (**9**) (100 mg, 0.61 mmol) was dissolved in THF (10 mL) and D_2O (5 mL), $LiOH{\bullet}H_2O$ (30 mg, 0.71 mmol) was added and the solution was stirred for 3 days. The solvent was removed to give **106** (102 mg, 100%): IR (KBr disk) 3059 (m br), 3021 (m), 1703 (s), 1406 (m), 1287 (s), 1269 (m), 1137 (m), 977 (m), 765 (m), 728 (m), 691 (s) cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) δ 7.2-7.1 (m, 5H, aryl-H), 6.5 (d, J = 15.9 Hz, 1H, CH=CHCD$_2$), 6.3 (d, J = 15.9 Hz, 1H, CH=CHCD$_2$); ^{13}C NMR (75 MHz, CD_3OD) δ 175.6 (CO acid), 138.5, 134.3, 129.8, 129.5, 128.5, 127.2, 126.4, 123.3 (aryl, vinyl C), 75.0 (CD$_2$); MS calcd for $C_{10}H_8D_2O_2$, 164.0806; found 164.0798 (44.7%), 119.0827 (100%).

***tert*-Butyl 4-(4-Nitrophenyl)-3-butenoate (107).**



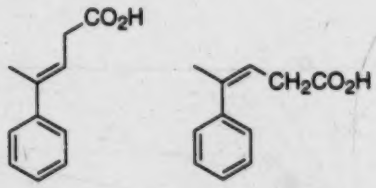
The literature procedure was modified.[23]: (4-Nitrophenyl)acetaldehyde (**49**) (100 mg, 0.5 mmol) was dissolved in THF (30 mL) and $Ph_3PCHCO_2{}^tBu$ (200 mg, 0.5 mmol) was added. The solution was stirred for 16 hours. The solvent was removed and the residue was purified by column chromatography (Florisil, 5% EtOAc in hexane to 50% EtOAc, Rf 0.7 (50% EtOAc) to yield the product **107** (70.0 mg, 53%): IR ($CHCl_3$ cast) 2979 (w), 2933 (w), 1728 (s), 1596 (m), 1517 (s), 1392 (w), 1343 (s), 1149 (s) cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 8.1 (d, J = 7.8 Hz, 2H, aryl-C<u>H</u> ortho to NO_2), 7.5 (d, J = 7.8 Hz, 2H, aryl-C<u>H</u> meta to NO_2), 6.5 (m, 2H, vinyl-C<u>H</u>), 3.2 (d, J = 7.2 Hz, 2H, C<u>H</u>$_2$), 1.5 (s, 9H, C(C<u>H</u>$_3$)$_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.0 (<u>C</u>O ester), 146.7, 143.4, 130.9, 127.7, 126.6, 123.8, 109.4 (aryl, vinyl <u>C</u>), 81.1 (<u>C</u>H$_2$), 39.4 (<u>C</u>(CH$_3$)$_3$), 27.9 (C(<u>C</u>H$_3$)$_3$); MS (EI) calcd for $C_{14}H_{17}NO_4$, 263.1158; found 263.1156 (0.83%), 57.0699 (100%); Anal. Calcd for $C_{14}H_{17}NO_4$: C, 63.86; H, 6.51; N, 5.32; found C, 63.81; H, 6.37; N, 5.22.

2-Carboxyethyl triphenylphosphonium chloride (108).[58]

The literature procedure was modified[58] A mixture of triphenylphosphine (52.4 g, 0.19 mol) and 3-chloropropionic acid (20.8 g, 0.19 mol) was heated to 150 °C for 2 hours. The

product, a glass, was dissolved in EtOH (400 mL) and crystallized by adding Et_2O (4 L). The solution was filtered to give **108** (47 g, 67%): mp 220-224 °C (lit. 221-223 °C)[58] IR ($CHCl_3$ cast) 3400-2900 (m, br), 1732 (s), 1437 (s), 1229 (m), 1188 (m), 1112 (m), 745 (m) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.8 (m, 15H, aryl-C<u>H</u>), 3.8 (m, 2H, C<u>H</u>$_2$CH$_2$CO$_2$H), 2.9 (m, 2H, CH$_2$C<u>H</u>$_2$CO$_2$H); MS (EI) calcd for $C_{18}H_{15}P$, 262.0911; found 262.0911 (100%), 73.0298 ($C_3H_5O_2$, 11.08%)

(3-*E*)-4-Phenyl-4-methyl-butenoic Acid (109a) and (3-*Z*)-4-phenyl-4-methyl-butenoic Acid (109b).

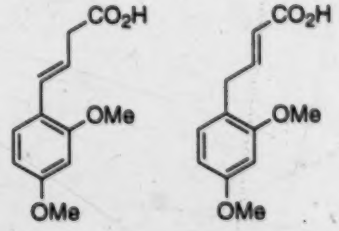


109a **109b**

The literature procedure was modified.[58] Hexamethyldisilazane (4.0 mL, 19.1 mmol) was dissolved in THF (50 mL) and the solution was cooled to 0°C and stirred under Ar. BuLi (11 mL, 1.75 M, 19 mmol) was added and the solution was stirred for 15 minutes. The solution was cooled to -78°C and compound **108** (3.2 g, 9.5 mmol) was added. The mixture was allowed to warm to -30°C and stirred for 45 minutes. The solution was then recooled to -78°C, acetophenone (1.13 g, 9.4 mmol) was added, and the mixture was allowed to warm to room temperature and stirred for 16 hours. The mixture was diluted with H_2O (150 mL) and washed with CH_2Cl_2 (50 mL). The aqueous phase was acidified to pH 2 with HCl (concentrated) and extracted with CH_2Cl_2 (3x100 mL). The organic extracts were dried ($MgSO_4$) and the solvent was removed. The residue was purified by chromatography (Florasil, 25% Et_2O in petroleum ether to 100% Et_2O, then 10% MeOH in Et_2O, R_f 0.3 in Et_2O) to give the product **109** as a 13:1 ratio of *E/Z* isomers (0.60 g,

36%): IR ($CHCl_3$ cast) 3400-2700 (br, m), 1731 (s), 1438 (s), 1188 (m), 1158 (m), 1114 (s), 723 (s) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$, **109a**) δ 10.1 (s, br, 1H, $CO_2\underline{H}$), 7.3 (m, 5H, aryl-C$\underline{H}$), 5.9 (d of t, J = 7.1, 1.3 Hz, 1H, vinyl-C$\underline{H}$ *E*), 3.3 (d of d, J = 7.1, 0.4 Hz, 2H, $C\underline{H}_2$), 2.1 (s, 3H, $C\underline{H}_3$); ^1H NMR (300 MHz, $CDCl_3$, **109b**) δ 10.1 (s, br, 1H, $CO_2\underline{H}$), 7.3 (m, 5H, aryl-C$\underline{H}$), 5.7 (d of t, J = 7.4, 1.4 Hz, 1H, vinyl-C$\underline{H}$ *Z*), 3.1 (d of d, J = 7.4, 1.1 Hz, 2H, $C\underline{H}_2$), 2.1 (s, 3H, $C\underline{H}_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 176 ($\underline{C}O$ acid), 135.2, 133.6, 132.0, 130.6, 128.6, 126.8, 125.7, 119.8, 118.2 (aryl, vinyl $\underline{C}$), 61.6 ($\underline{C}H_2$), 34.4 ($\underline{C}H_3$); MS (EI) calcd for $C_{11}H_{12}O_2$, 176.0837; found 176.0836 (30.6%) 105.0592 (100%).

4-(2,4-Dimethoxyphenyl)-3-butenoic Acid (110a) and 4-(2,4-Dimethoxy phenyl)-2-butenoic Acid (110b).

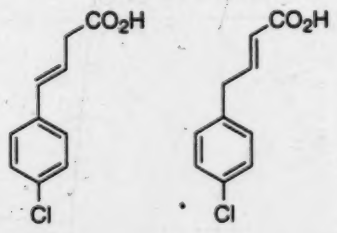


110a **110b**

The method used to synthesize **109a** and **b** was employed to transform **108** (3.2 g, 9.5 mmol) and 2,4 dimethoxybenzaldehyde (1.65 g, 9.90 mmol) to **110a** and **110b.** A portion of the product (25%) was purified by reverse phase HPLC (Resolve, RP C-18, monitored at 254 nm); solvent A: 0.1% TFA in H_2O, solvent B 0.1% TFA in MeCN; starting conditions 70/30 A/B changing to 40/60 A/B over 20 minutes, holding for 5 minutes, reverting to 70/30 A/B in 5 minutes and holding for 5 minutes. The product, a 98:2 ratio of **110 a** and **b** (312 mg, 60%) had a retention time of 18 minutes: IR ($CHCl_3$ cast) 3004 (m, br), 1771 (m), 1732 (m), 1612 (m), 1589 (m), 1508 (s), 1290 (m), 1209 (s), 1158 (m),

1032 (m) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$, **110a**) δ 8.4 (s, br, 1H, $CO_2\underline{H}$), 7.4 (d, J = 7.2 Hz, 1H, aryl-C$\underline{H}$ meta to OMe), 6.8 (d, J = 15.9 Hz, 1H, C$\underline{H}$=CHCH$_2$), 6.5 (d of d, J = 8.4, 2.5 Hz, 1H, aryl-C$\underline{H}$ ortho to OMe), 6.4 (d, J = 2.3 Hz, 1H, aryl-C$\underline{H}$ ortho to OMe), 6.2 (d of t, J = 15.9, 7.1 Hz, 1H, CH=C$\underline{H}$CH$_2$), 3.8 (s, 6H, OC$\underline{H}_3$), 3.3 (d, J = 7.1 Hz, 2H, C$\underline{H}_2$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 163.0 ($\underline{C}$O), 132.8, 128.0, 115.7, 115.4, 115.2, 114.8, 105.0 (aryl $\underline{C}$, vinyl $\underline{C}$), 60.2, 59.8, 55.8 ($\underline{C}$ alkyl); MS calcd for $C_{12}H_{14}O_4$, 222.0892; found 222.0920 (12.3%), 167.0708 (100%).

4-(4-Chlorophenyl)-3-butenoic Acid (111a) and 4-(4-Chlorophenyl)-2-butenoic Acid (111b).[71]

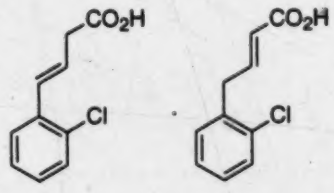


111a **111b**

The method used to synthesize **109a** and **b** was employed to transform **108** (1.6 g, 4.8 mmol) and 4-chlorobenzaldehyde (0.66 g, 4.7 mmol) to **111a** and **111b.** The product was purified by HPLC in the same manner as **110a** and **b**. The product, a 95:5 ratio of **111a** and **111b** (166 mg, 72%) retention time of 10.5 minutes: IR ($CHCl_3$ cast) 3400-2900 (br, w), 1716 (s), 1492 (m), 1399 (m), 1226 (m), 1161 (w), 1087 (m), 1012 (w), 975 (m), 793 (m) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$, **111a**) 8.5 (s, br, 1H, $CO_2\underline{H}$), 7.3 (s, 4H, aryl-C$\underline{H}$), 6.5 (d, J = 15.9 Hz, 1H, C$\underline{H}$=CHCH$_2$), 6.3 (d of t, J = 15.9, 7.0 Hz, 1H, CH=C$\underline{H}$CH$_2$), 3.3 (d, J = 7.0 Hz, 2H, C$\underline{H}_2$); ^{13}C NMR (75 MHz, $CDCl_3$) 177.0 ($\underline{C}O_2$H), 135.1 (aryl-$\underline{C}$ Cl attached), 132.8 ($\underline{C}$H meta to Cl), 130.1 (aryl-$\underline{C}$ alkene

attached), 128.7 (vinyl-CH), 127.5 (aryl-CH ortho to Cl), 121.5 (vinyl-CH), 37.8 ($\underline{C}H_2$); MS (EI) calcd for $C_{10}H_9O_2{}^{35}Cl$, 196.0291; found 196.0291 (100%).

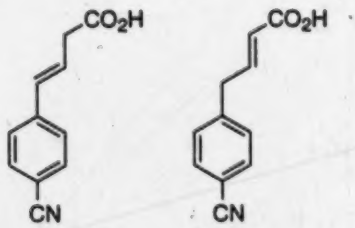
4-(2-Chlorophenyl)-3-butenoic Acid (112a) and 4-(2-Chlorophenyl)-2-butenoic Acid (112b).



112a **112b**

The method used to synthesize **109a** and **b** was employed to transform **108** (1.6 g, 4.8 mmol) and 2-chlorobenzaldehyde (0.66 g, 4.7 mmol) to **112a** and **112b.** The product was purified by HPLC in the same manner as **110a** and **b**. The product, a 9:1 ratio of **112a** and **112b** (145 mg, 65%) had a retention time of 18 minutes: IR ($CHCl_3$ cast) 3300-2600 (br, m), 1707 (m), 1316 (m), 1271 (m), 1122(s), 768 (s) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$, **112a**) δ 9.7 (s br, 1H, $CO_2\underline{H}$), 7.6 (d of d, J = 7.4, 2.1 Hz, 1H, aryl-C$\underline{H}$ ortho to Cl), 7.4 (d of d, J, = 9.2, 1.5 Hz, 1H, ortho to alkene), 7.2 (m, 2H, aryl-C$\underline{H}$ meta and para to Cl), 6.9 (d, J = 15.8, 1H, C$\underline{H}$=CHCH$_2$), 6.3 (d of t, J = 15.8, 7.3 Hz, 1H, CH=C$\underline{H}$CH$_2$), 3.4 (d of d, J = 7.3, 1.6 Hz, 2H, CH=CHC$\underline{H}_2$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 177.4 ($\underline{C}O_2H$), 148.0, 131.9, 129.9, 127.5, 127.1, 125.8, 125.7, 122.0 (aryl $\underline{C}$, vinyl $\underline{C}$), 38.0 ($\underline{C}H_2$); MS (EI) calcd for C_9H_8Cl, 151.0345; found 151.0346 (M-CO_2H).

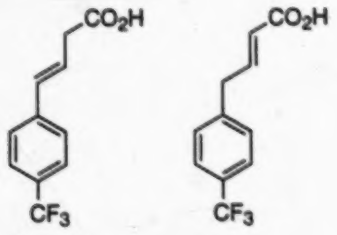
4-(4-Cyanophenyl)-3-butenoic Acid (113a) and 4-(4-Cyanophenyl)-2-butenoic Acid (113b).[71]



113a **113b**

The method used to synthesize **109a** and **b** was employed to transform **108** (0.19 g, 0.56 mmol) and 4-cyanobenzaldehyde (0.10 g, 0.56 mmol) to **113a** and **113b.** The product was purified by HPLC in the same manner as **110a** and **b**. The product, a 1:1 ratio of **113a** and **b** (73 mg, 70%) had a retention time of 11 minutes: IR ($CHCl_3$ cast) 3400-2900 (m, br), 2229 (m), 1716 (s), 1683 (s), 1436 (m), 1282 (m), 1156 (m), 1129 (m), 1093 (m), 760 (m) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$, **113a**) δ 7.5 (m, 4H, aryl-C<u>H</u>), 6.5 (d, J = 15.9, 1H, C<u>H</u>=CHCH$_2$), 6.4 (d of t, J = 15.9, 6.9 Hz, 1H, CH=C<u>H</u>CH$_2$), 3.2 (d, J = 6.8 Hz, 1H, C<u>H</u>$_2$); ^1H NMR (300 MHz, $CDCl_3$, **113b**) δ 7.5 (m, 4H, aryl-C<u>H</u>), 6.9 (d of t, J = 15.9, 6.8 Hz, 1H, CH$_2$C<u>H</u>=CH), 5.8 (d, J = 15.9, 0.5H, CH$_2$CH=C<u>H</u>), 3.3 (d of d, 7.0, 1.5 Hz, 1H, C<u>H</u>$_2$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173 (<u>C</u>O$_2$H), 129.1, 129.0, 128.4, 127.9, 126.9, 126.3, 122.2, 121.7 (<u>C</u>, aryl, <u>C</u> vinyl, <u>C</u>N), 35.5 (<u>C</u>H$_2$ isomer), 35.2 (<u>C</u>H$_2$ isomer); MS (EI) calcd for $C_{11}H_9O_2N$, 187.0633; found 187.0632 (33%), 142.0654 (100%).

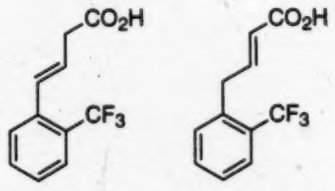
4-(4-(Trifluoromethyl)phenyl)-3-butenoic Acid (114a) and 4-(4-(Trifluoro methyl)phenyl)-2-butenoic Acid (114b).[70]



114a **114b**

The method used to synthesize **109a** and **b** was employed to transform **108** (1.6 g, 4.8 mmol) and 4-(trifluoromethyl)benzaldehyde (0.82 g, 0.63 mL, 4.7 mmol) to **114a** and **114b.** The product was purified by HPLC in the same manner as **110a** and **b.** The product, a 9:1 ratio of **114a** and **114b** (250 mg, 46%) had a retention time of 17.5 minutes: IR (CH_2Cl_2 cast) 3416 (s, br), 2926 (w), 2354 (w), 1559 (s), 1411 (s), 1325 (s), 1161 (s), 1121 (s), 1067 (s), 1016 (s), 966 (s), 857 (w), 811 (w), 684 (m) cm^{-1}; ^1H NMR (360 MHz, CD_3OD, **114a**) δ 7.5 (d, J = 9 Hz, 2H, aryl-C<u>H</u>), 7.5 (d, J = 9 Hz, 2H, aryl-C<u>H</u>), 6.6 (d, J = 16.0, 1H, C<u>H</u>=CHCH$_2$), 6.5 (d of t, J = 16.0, 6.7 Hz, 1H, CH=C<u>H</u>CH$_2$), 3.3 (d, J = 6.3 Hz, 2H, C<u>H$_2$</u>); ^{13}C NMR (100 MHz, CD_3OD) δ 177.3 (<u>C</u>O acid), 152, 143, 130.7, 130.2, 129.7, 128.3, 126.8 (aryl, vinyl <u>C</u>), 37.3 (<u>C</u>H$_2$); MS (EI) calcd for $C_{11}H_9O_2F_3$, 230.0555; found 230.0556 (51.93%), 185.0577 (100%).

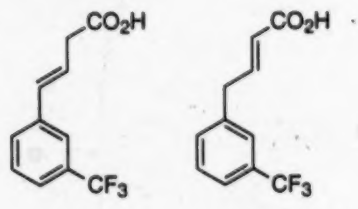
4-(2-(Trifluoromethyl)phenyl)-3-butenoic Acid (115a) and 4-(2-(Trifluoro methyl)phenyl)-2-butenoic Acid (115b).



115a **115b**

The method used to synthesize **109a** and **b** was employed to transform **108** (1.6 g, 4.8 mmol) and 2-(trifluoromethyl)benzaldehyde (0.82 g, 0.63 mL, 4.7 mmol) to **115a** and **115b.** The product was purified by HPLC in the same manner as **110a** and **b**. The product, a 9:1 ratio of **115a** and **115b** (200 mg, 37%) had a retention time of 17.5 minutes: IR ($CHCl_3$ cast) 3400-2900 (br, m), 1711 (s), 1314 (s), 1278 (m), 1160 (m), 1121 (s), 1035 (m), 766 (m) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$, **115a**) δ 7.8 (m, 1H, aryl C<u>H</u> ortho to CF_3), 7.7 (m, 1H, aryl C<u>H</u> ortho to vinyl), 7.5 (m, 1H, arylC<u>H</u> meta to CF_3), 7.4 (m, 1H, aryl C<u>H</u> para to CF_3), 7.0 (d, J = 15.8 Hz, 1H, C<u>H</u>=CHCH$_2$), 6.4 (d of t, J = 15.8, 7.2 Hz, 1H, CH=C<u>H</u>CH$_2$), 3.4 (d of d, J = 7.2, 1.4, 2H, C<u>H</u>$_2$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.8 (<u>C</u>O acid), 131.9, 130.9, 129.8, 129.0, 128.9, 127.5, 127.4, 125.7, 125.3 (aryl, vinyl <u>C</u>), 37.9 (<u>C</u>H$_2$); MS (EI) calcd for $C_{11}H_9O_2F_3$, 230.0555; found 230.0554 (100%).

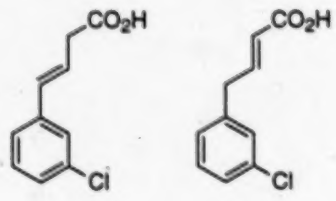
4-(3-(Trifluoromethyl)phenyl)-3-butenoic Acid (116a) and 4-(3-(Trifluoro methyl)phenyl)-2-butenoic Acid (116b).



116a **116b**

The method used to synthesize **109a** and **b** was employed to transform **108** (1.6 g, 4.8 mmol) and 3-(trifluoromethyl)benzaldehyde (0.82 g, 0.66 mL, 4.7 mmol) to **116a** and **116b.** The product was purified by HPLC in the same manner as **110a** and **b**. The product, a 8:1 ratio of **116a** and **b** (225 mg, 42%) had a retention time of 17 minutes: IR ($CHCl_3$ cast) 3400-2900 (m, br), 1736 (s), 1709 (m), 1438 (w), 1330 (s), 1273 (m), 1202 (m), 1164 (s), 1124 (s), 1072 (m) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$, **116a**) δ 7.5 (m, 4H, aryl-C<u>H</u>), 6.5 (d, J = 16.0 Hz, 1H, C<u>H</u>=CHCH$_2$), 6.3 (d of t, J = 16.0, 7.1, 1H, CH=C<u>H</u>CH$_2$), 3.3 (d of d, J = 7.1, 1.1 Hz, 2H, C<u>H</u>$_2$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 137.4, 132.7, 131.1, 129.0, 128.3, 127.8, 124.3, 124.2, 123.0, 37.6; MS (EI) calcd for $C_{11}H_9O_2F_3$, 230.0555; found 230.0554 (94%).

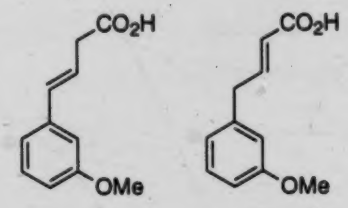
4-(3-Chlorophenyl)-3-butenoic Acid (117a) and 4-(3-(Chlorophenyl)-2-butenoic Acid (117b).



117a **117b**

The method used to synthesize **109a** and **b** was employed to transform **108** (1.6 g, 4.8 mmol) and 3-chlorobenzaldehyde (0.66 g, 0.53 mL, 4.7 mmol) to **117a** and **117b.** The product was purified by HPLC in the same manner as **110a** and **b**. The product, a 10:1 ratio of **117a** and **b** (240 mg, 64%) had a retention time of 16 minutes: IR ($CHCl_3$ cast) 3300-2700 (br, m), 1709(s), 1655 (m), 1593 (m), 1438 (m), 1196 (m), 1165 (m), 1112 (m), 777 (m) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$, **117a**) δ 9.6 (s, br, 1H, $CO_2\underline{H}$), 7.7 (m, 2H, aryl-C<u>H</u> ortho and meta to Cl), 7.2 (m, 2H, aryl-C<u>H</u> ortho to alkene), 6.3 (d, J = 16.0 Hz, 1H, C<u>H</u>=CHCH$_2$), 6.2 (d of t, J = 16.0, 6.9 Hz, 1H, CH=C<u>H</u>CH$_2$), 3.2 (d, J = 6.9 Hz, 2H, C<u>H</u>$_2$); 13C NMR (75 MHz, $CDCl_3$) δ 176.4 (<u>C</u>O acid), 138.6, 135.2, 132.2, 130.7, 127.4, 126.1, 124.8, 123.0 (<u>C</u> aryl and vinyl), 38.0 (<u>C</u>H$_2$); MS (EI) calcd for $C_{10}H_9O_2Cl$, 196.0291; found 196.0288 (71%), 115.0549 (100%).

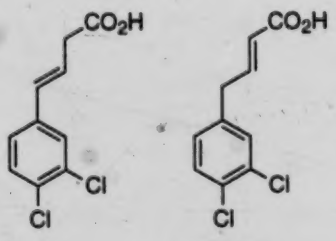
4-(3-Methoxyphenyl)-3-butenoic Acid (118a) and 4-(3-(Methoxy phenyl)-2-butenoic Acid (118b).[71]



118a **118b**

The method used to synthesize **109a** and **b** was employed to transform **108** (1.6 g, 4.8 mmol) and 3-methoxybenzaldehyde (0.64 g, 0.57 mL, 4.7 mmol) to **118a** and **118b.** The product was purified by HPLC in the same manner as **110a** and **b**. The product, a 6:1 ratio of **118a** and **118b** (161 mg, 43%) had a retention time of 11 minutes: IR ($CHCl_3$ cast) 3400-2800 (br, m) , 1709 (s) 1598 (s), 1435 (m), 1289 (m), 1156 (s), 776 (m) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$, **118a**) δ 9.2 (s, br, 1H, $CO_2\underline{H}$), 7.2 (t, J = 8.0 Hz, 1H, aryl-C$\underline{H}$ meta to OMe), 7.1 (d, J = 7.6 Hz, 1H, aryl-C$\underline{H}$ ortho to OMe), 7.0 (s, 1H, aryl-C$\underline{H}$ ortho to OMe), 6.5 (d, J = 16.0 Hz, 1H, C$\underline{H}$=CHCH$_2$), 6.3 (d of t, J = 16.0, 6.5 Hz, 1H, CH=C$\underline{H}$CH$_2$), 3.3 (d of d J = 6.5, 1.1 Hz, 2H, CH=CHC$\underline{H}_2$); ^1H NMR (300 MHz, $CDCl_3$, **118b**) δ 9.2 (s, br, 1H, $CO_2\underline{H}$), 7.2 (t, J = 8.0 Hz, 1H, aryl-C$\underline{H}$ meta to OMe), 7.1 (d, J = 7.6 Hz, 1H, aryl-C$\underline{H}$ ortho to OMe), 7.0 (s, 1H, aryl-C$\underline{H}$ ortho to OMe), 6.8 (d of t, J = 15.9, 6.7 Hz, 1H, CH$_2$C$\underline{H}$=CH), 5.8 (d, J = 15.9 Hz, 1H, CH$_2$CH=C$\underline{H}$), 3.2 (d J = 6.7 Hz, 0.3H, CH=CHC$\underline{H}_2$); ^{13}C (75 MHz, $CDCl_3$) δ 177.4 ($\underline{C}O_2H$), 159.7 (aryl-$\underline{C}$ OMe attached), 138 (aryl-$\underline{C}$ alkene attached), 133.7 (vinyl-$\underline{C}$H), 129.4 (aryl-$\underline{C}$ meta to OMe), 122.7 (aryl-$\underline{C}$ ortho to alkene), 119 (aryl-$\underline{C}$ ortho to OMe), 113.3 (vinyl-$\underline{C}$H), 111.6 (aryl-$\underline{C}$ ortho to alkene), 55.1 ($\underline{C}H_3$), 38.0 ($\underline{C}H_2$); MS (EI) calcd for $C_{11}H_{10}O_3$, 174.0680; found 174.0681 (M-H_2O, 4.5%), 136.0521 (100%).

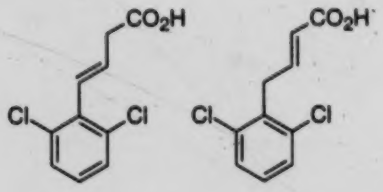
4-(3,4-Dichlorophenyl)-3-butenoic Acid (119a) and 4-(3,4-(Dichlorophenyl)-2-butenoic Acid (119b).[71]



119a **119b**

The method used to synthesize **109a** and **b** was employed to transform **108** (1.6 g, 4.8 mmol) and 3,4-dichlorobenzaldehyde (0.82 g, 4.7 mmol) to **119a** and **119b.** The product was purified by HPLC in the same manner as **110a** and **b**. The product, a 15:1 ratio of **119a** and **119b** (235 mg, 49%) had a retention time of 20 minutes: IR ($CHCl_3$ cast) 3500-2800 (m, br), 1708 (s), 1473 (m), 1438 (m), 1266 (w), 1194 (m), 1134 (m), 1121 (m), 724 (m) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$, **119a**) δ 9.2 (s, br, 1H, $CO_2\underline{H}$), 7.6 (m, 1H, aryl-C<u>H</u>, ortho to Cl), 7.5 (d, J = 2.0 Hz, 1H, aryl-C<u>H</u>, ortho to alkene), 7.3 (d of d, J = 8.4, 2.0 Hz, 1H, aryl-C<u>H</u> ortho to alkene), 6.4 (d, J = 16.0 Hz, 1H, C<u>H</u>=CHCH$_2$), 6.3 (d of t, J = 16.0, 6.9 Hz, 1H, CH=C<u>H</u>CH$_2$), 3.3 (d of d, J = 6.9, 1.0 Hz, 2H, C<u>H</u>$_2$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.2 (<u>C</u>O acid), 136.8, 135.5, 133.3, 132.5, 132.0, 131.4, 130.6, 123.4 (<u>C</u> aryl and vinyl), 37.8 (<u>C</u>H$_2$); MS (EI) calcd for $C_{10}H_8O_2{}^{35}Cl_2$, 229.9901; found 229.9887 (60%), 184.99234 (100%).

4-(2,6-Dichlorophenyl)-3-butenoic acid (120) and 4-(2,6-(dichlorophenyl)-2-butenoic acid (121).



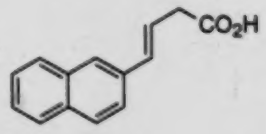
120 **121**

The method used to synthesize **109a** and **b** was employed to transform **108** (1.6 g, 4.8 mmol) and 2,6-dichlorobenzaldehyde (0.82 g, 4.7 mmol) to **120** and **121.** The product was purified by HPLC in the same manner as **110a** and **b**. Two peaks were collected. The first a 15:1 ratio of **120** and **121** (175 mg, 40%) had a retention time of 18 minutes and the second, a 1:9 ratio of **120** and **121** (50 mg, 12%) had a retention time of 19 minutes. Compound **120**: IR ($CHCl_3$ cast) 3300-2700 (br, m), 1695 (s), 1645 (m), 1435 (m), 1277 (m), 1231 (m), 779 (m) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.4 (m, 2H, aryl-C<u>H</u> ortho to Cl), 7.2 (m, 1H, aryl-C<u>H</u> meta to Cl), 6.4 (d, J = 16.3 Hz, 1H, C<u>H</u>=CHCH$_2$), 6.2 (d of t, J = 16.3, 7.2 Hz, 1H, CH=C<u>H</u>CH$_2$), 3.1 (d of d, J = 7.1, 1.4 Hz, 2H, CH=CHC<u>H</u>$_2$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 177.4 (<u>C</u>O, acid), 145.4, 135.7, 134.7, 129.0, 128.4, 127.9, 126.9, 121.9 (aryl, vinyl <u>C</u>) 34.2 (<u>C</u>H$_2$); MS (EI) calcd for $C_{10}H_8O_2{}^{35}Cl_2$, 229.9901; found 229.9877 (100%); Anal. Calcd for $C_{10}H_8O_2Cl_2$: C, 51.98; H, 3.49; Cl, 30.68; found C, 51.70; H, 3.35.

Compound **121**: IR ($CHCl_3$ cast) 3300-2800 (br, m), 1708 (s), 1642 (w), 1423 (m), 1381 (w), 1221 (m), 749 (m) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.4 (m, 2H, aryl-C<u>H</u> ortho to Cl), 7.2 (m, 1H, aryl-C<u>H</u> meta to Cl), 7.1 (d of t, J = 15.7, 6.2 Hz, 1H, CH$_2$C<u>H</u>=CH), 5.7 (d of t, J = 15.7, 1.7 Hz, 1H, CH$_2$CH=C<u>H</u>), 3.9 (d of d, J = 6.2, 1.7, 2H, C<u>H</u>$_2$CH=CH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.6 (<u>C</u>O, acid), 145.3, 135.7,

133.8, 129.8, 128.7, 128.6, 128.3, 121.9 (aryl, vinyl C) 33.7 ($\underline{C}H_2$); MS (EI) calcd for $C_{10}H_8O_2{}^{35}Cl_2$, 229.9901; found 229.9892 (100%) Anal. Calcd for $C_{10}H_8O_2Cl_2$: C, 51.98; H, 3.49; Cl, 30.68; found C, 51.78; H, 3.36.

4-(2-Naphthyl)-3-butenoic Acid (122).



The method used to synthesize **109a** and **b** was employed to transform **108** (1.6 g, 4.8 mmol) and 2-naphthaldehyde (0.73 g, 4.7 mmol) to **122.** The product was purified by HPLC in the same manner as **110a** and **b**, retention time of 24 minutes: IR ($CHCl_3$ cast) 3500-2900 (br, m), 1734 (s), 1436 (m), 1269 (m), 1168 (s), 747 (m) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.8 (m, 3H, α-C$\underline{H}$), 7.7 (s, 1H, C$\underline{H}$ ortho to alkene), 7.5 (m, 2H, β-C$\underline{H}$), 6.7 (d, J = 15.9 Hz, 1H, C$\underline{H}$=CHCH$_2$), 6.5 (d of t, J = 15.9, 7.0 Hz, 1H, CHC$\underline{H}$CH$_2$), 3.3 (d of d, J = 7.0, 1.4 Hz, 2H, C$\underline{H}_2$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 134.3 (aryl-$\underline{C}$ alkene attached), 133.6 (vinyl-$\underline{C}$H), 133.5 (aryl-$\underline{C}$), 128.1, 128.0, 127.7 (α-$\underline{C}$H), 126.3, 126.1, 125.9 (β-$\underline{C}$H), 123.5 (α-$\underline{C}$ ortho to alkene), 122.0 (vinyl-$\underline{C}$H), 38.3 ($\underline{C}H_2$); MS (EI) calcd for $C_{13}H_{11}$, 167.0861; found 167.0860 (M^+-CO_2H, 100%).

Isolation of Peptidylglycine α-Hydroxylating Monooxygenase (PHM) from Heads of Honey Bees (*Apis mellifera*)

PHM Assays: An HPLC assay similar to Jones *et al.*[73] was used to determine the levels of PHM activity, using Dansyl-L-Phe-L-Phe-Gly (**42**) as substrate (53 μM in 40 μL total volume, 1 mM ascorbate, 5 μM copper sulfate, 25 mM potassium iodide, and 0.125 mg/mL catalase) along with an appropriate amount of enzyme solution in a total volume of 125 μL. After incubation for 1 hour at 37°C, the enzymatic reaction was quenched with 30 μL of 2 M NaOH to hydrolyze the α-hydroxyglycine peptide. The mixture was shaken on a vortex mixer and returned to the 37°C bath for 5 minutes to ensure total hydrolysis. The mixture was then acidified with 32.5 μL of 2 M HCl, vortexed, and the peptide amide was separated by HPLC (Waters Radial Pak, 8x100 mm Nova-Pak C_{18} (4 μm) column; mobile phase A: 28 mM NaOAc, pH 5.5, B: MeCN; 45% B for 4 minutes, linear gradient to 75% B in 3 minutes, hold for 1 minute then return to 45% B in 2 minutes; flow rate of 1.5 mL/min; UV detection at 250 nm). The amount of *C*-terminal peptide amide was quantified by comparing the integrated HPLC peak area with a standard curve constructed from serial dilutions of an authentic sample of Dansyl-L-Phe-L-Phe-NH_2 (**43**).

Isolation of PHM: All steps were performed at 4°C unless otherwise indicated. Frozen honey bees (-70°C) were agitated to separate the heads from other body parts. A typical isolation began with the homogenization of approximately 10 g of bee heads in 100 mL of cold 50 mM NaH_2PO_4/Na_2HPO_4, 200 mM NaCl, pH 6.8 buffer (buffer A) using an ultra-turrax tissue homogenizer for 3x30 second intervals with a 1 minute cooling period between each interval. This suspension was centrifuged at 27000 g for 30 minutes. The crude pellet was resuspended in 50 mL of buffer A using a Potter-Elvehjem tissue grinder and was diluted with an equal volume of buffer A containing 2% n-octyl-β-D-glucopyranoside, thereby bring the final concentration of detergent to 1%. This suspension was stirred at 4°C for 1.5 hours and then centrifuged at 27000 g for 1 hour, the supernatant

was made 55% saturated in $(NH_4)_2SO_4$ over the course of 1 hour and then equilibrated for an additional 30 minutes. After centrifugation at 27000 g for 1 hour, the supernatant was discarded and the pellet was resuspended in 40 mL of 50 mM NaH_2PO_4/Na_2HPO_4, 500 mM NaCl, pH 6.8 buffer (buffer B), frozen, and stored at -70°C overnight. This ammonium sulfate fraction was thawed and centrifuged (27000 g, 15 minutes) and the supernatant was applied to a 10 mL column of chelating Sepharose 6B (Pharmacia) charged with Cu^{2+}. The flow rate was 0.5 mL/minute. The column was washed with 40 mL of buffer B, and then bound protein was eluted using a linear gradient of buffer B to 50 mM imidazole hydrochloride in buffer B, pH 7.0. Each reservoir contained 200 mL of the respective buffer, and 5 mL fractions were collected. Fractions containing amidating activity totalled 90 mL and were pooled and concentrated to 20 mL with an Amicon (Beverly, MA) ultrafiltration apparatus fitted with a YM-10 membrane. This material was frozen and stored at -70°C overnight. After thawing, the material from the Cu-chelating column was applied to a substrate affinity column, prepared by coupling 85 μmol of L-Phe-L-Phe-Gly (**6**) to 6 mL of Affi-Gel 15 (Bio-Rad) following the manufacturers directions. The loading flow rate was 15 mL/hour, and the unretained material was recirculated once. After collecting the unretained protein a second time, the column was washed with buffer A until the A_{280} returned to zero, and then bound protein was eluted with 1 M imidazole, pH 8.5. Four fractions (4 mL) were collected, and the imidazole was immediately removed by a repetitive series (three times) of concentration/dilution steps using an Amicon Centricon-30 ultrafiltration device. Final volumes were approximately 0.5 mL. Protein content was determined relative to bovine serum albumin using the Bio-Rad protein assay kit (Bradford, 1976).[74] The enzyme solution was stored at -70°C and remained active for at least eight weeks under these conditions. If the solution is kept at 4°C, there is little activity loss for at least 48 hours.

Table 11: Protein and Activity Assays of Enzyme Isolation

Fraction	Amount Added to assay (μL)	Protein Content μg/mL	Activity (pmol/μg/h)
Affinity (Pooled) (4 mL)	5	15.8	288
Copper (Pooled) (20 mL)	25	174.9	305
Ammonium Sulfate Precipitate (supernatant)	25	372.5	3.6
Ammonium Sulfate Precipitate (pellet) (40 mL)	25	444.7	5.4
Detergent (supernatant) (100 mL)	25	407.8	21.5
Detergent (pellet)	25	247.6	24.7
Affinity column (unretained)	25	33.2	3.5
Copper column (unretained)	25	33.7	1.5

Bulk Inhibition Study of PAM (from Rat Thyroid Carcinoma (RTC)) with (4-[4-[*N*-(6-((*N*-Dansyl)amino)hexanoyl)amino]phenyl]-3-butenoic Acid (78).

PAM (3 mg, 40 nmol) was inhibited with the dansyl labelled phenylbutenoic acid **78** (0.64 mg, 1.6 μmol) in 5 mL of cocktail (1 mM ascorbate, 5 μM copper sulfate, 25 mM potassium iodide, and 0.125 mg/mL catalase, 50 mM NaH_2PO_4/Na_2HPO_4, 200 mM NaCl, pH 6.8 buffer). The enzyme solution was incubated overnight at 37°C. Aliquots (60 μL) were taken every hour for 6 hours, substrate (**42**) was added (5 μL, 53 μM), and the solution was asayed for enzyme activity. Activity was absent by the end of 6 hours.

Digestion of PAM with Endoproteinase Arg-C: The buffer of the inhibited enzyme was changed to 5 mM $CaCl_2$, 0.1 M NH_4HCO_3 pH 8 by a repetitive series (ten times) of concentration/dilution steps using an Amicon Centricon-30 ultrafiltration device. The final volume was 1.5 mL. Endoproteinase Arg-C (dissolved in 1 mL of digestion buffer) was added (50:1 ratio of PAM to digestion enzyme)and the solution was incubated at room temperature for 3 days. A control experiment was also completed in which uninhibited PAM (100 μg, RTC) was digested in the same manner.

Purification of Digested PAM Inhibited with Dansyl Derivative 78: The digestion mixture was purified by HPLC (Delta pak protein column, 300 Å, C4, 8 mm x 10 cm; mobile phase A: 0.1% TFA in water, B: 80/20/0.1 MeCN/H_2O/TFA; 100% A, linear gradient to 100% B in 100 minutes; flow rate of 1.5 mL/min; UV detection at 250 nm). Three peaks were collected with retention times of 18, 27, and 73.5 minutes.

Bulk Inhibition Study of PAM (RTC) with (4-[4-[*N*-(6-((*N*-Biotin)amino) hexanoyl)amino]phenyl]-3-butenoic Acid 80.

PAM (3 mg, 40 nmol) was inhibited with the biotin derivative **80** (0.96 mg, 2.4 μmol) in 10 mL of cocktail (0.75 mM DMPD•HCl, 16 μM copper sulfate, and 1 mg/mL catalase, 50 mM NaH_2PO_4/Na_2HPO_4, 200 mM NaCl, pH 8 buffer). The enzyme solution was incubated overnight at 37°C. Aliquots (60 μL) were taken every 15 minutes for 2 hours, substrate **42** was added (5 μL, 53 μM), and enzyme activity was determined by monitoring the absorbance at 515 nm. Activity was essentially gone by the end of 1 hour.

Digestion of PAM (RTC) with Endoproteinase Arg-C Inhibited with Biotin Derivative 80 : The buffer of the inhibited enzyme was changed to 5 mM NaCl, 0.1 M NaH_2PO_4/Na_2HPO_4, pH 8 by a repetitive series (ten times) of concentration/dilution steps using an Amicon Centricon-30 ultrafiltration device. The final volume was 1.5 mL. Endoproteinase Arg-C (dissolved in 1 mL of digestion buffer) was added (50:1 ratio of

PAM to digestion enzyme) and the solution was incubated at room temperature for 3 days. The digestion enzyme was then inactivated by heating at 90°C for 10 minutes followed by sonication for 3 hours.

Digestion of PAM with Endoproteinase Asp-N Inhibited with Biotin derivative 80: The buffer of the inhibited enzyme was changed to 5 mM NaCl, 0.1 M NaH_2PO_4/Na_2HPO_4, pH 5.5 by a repetitive series (ten times) of concentration/dilution steps using an Amicon Centricon-30 ultrafiltration device. The final volume was 1.5 mL. Endoproteinase Asp-N (dissolved in 1 mL of digestion buffer) was added (100:1 ratio of PAM to digestion enzyme). The solution was incubated at room temperature for 3 days and the digestion enzyme was then inactivated by sonication for 4 hours.

Purification of Digested PAM Inhibited with Biotin Derivative 80: The digestion mixture from the endoproteinase Arg-C digestion was applied to an avidin column (3 mL of gel) (Pierce) following the standard protocols provided by the company. The column was prepared by washing the beads with 20 mM NaH_2PO_4/Na_2HPO_4, 500 mM NaCl, pH 7.5 (3x5 mL), then 10 mL of biotin buffer (1 mg/mL biotin in 20 mM NaH_2PO_4/Na_2HPO_4, 500 mM NaCl, pH 7.5), followed by an additional wash with 20 mM NaH_2PO_4/Na_2HPO_4, 500 mM NaCl, pH 7.5 (3x5 mL). The biotin was eluted with 2 x 10 mL of the glycine elution buffer (0.1 M glycine•HCl, pH 2.2) followed by a final wash with 20 mM NaH_2PO_4/Na_2HPO_4, 500 mM NaCl, pH 7.5 (3x5 mL). The sample was applied in a 1.5 mL aliquot of 20 mM NaH_2PO_4/Na_2HPO_4, 500 mM NaCl, pH 7.5 buffer and the beads were agitated for 1 hour at 4°C. The beads were washed with binding buffer (20 mM NaH_2PO_4/Na_2HPO_4, 500 mM NaCl, pH 7.5; 10x5 mL) and H_2O (5x5 mL) to remove the unbound peptides. The bound peptides were eluted with 8 M guanidine•HCl, pH 1, 10 mL of elution buffer was added and the beads were agitated for 15 minutes, this process was repeated 2 times. The eluted peptides were desalted using a C-18 sep pak. The eluted peptides were applied to the column and recycled 5 times to

ensure total binding. The column was washed with 20 mL of H_2O and then eluted with 20 mL of MeCN.

The digestion mixture was purified by HPLC (Vydac column, C18, ; mobile phase A: 0.1% TFA, B: 80/20/0.1 MeCN/H_2O/TFA; 100% A, linear gradient to 100% B in 60 minutes; flow rate of 1.0 mL/min; UV detection at 220 nm). Three peaks were collected with retention times of 49, 51, and 53 minutes, and these were lyophilized.

References

1. Eipper, B. A.; Stoffers, D. A.; Mains, R. E. *Annu. Rev. Neurosci.* **1992**, *15*, 57-85.
2. Eipper, B. A.; Smyth, D. G. *Annu. Rev. Physiol.* **1988**, *50*, 333-344.
3. Bradbury, A. F.; Mistry, J.; Smyth, D. G. *Eur. J. Biochem.* **1990**, *189*, 363-368.
4. Bradbury, A. F.; Finnie, M. D. A.; Smyth, D. G. *Nature* **1982**, *298*, 686-688.
5 Zabriskie, T. M.; Cheng, H.; Vederas, J. C. *J. Chem. Soc., Chem. Commun.* **1991**, 571-572.
6. Ramer, S. E.; Cheng, H.; Palcic, M. M.; Vederas, J. C. *J. Am. Chem. Soc.* **1988**, *110*, 8526-8532.
7. a) Kawahara, T.; Suzuki, K.; Iwasaki, Y.; Shimoi, H.; Akita, M.; Moro-Oka, Y; Nishikawa, Y. *J. Chem. Soc., Chem. Commun.* **1992**, 625-626.
   b) Ping, D.; Katopodis, A.; May, S. W. *J. Am. Chem. Soc.* **1992**, *114*, 3998-4000.
8. Noguchi, M.; Seino, H.; Kochi, H.; Okamoto, H.; Tanaka, T.; Hirama, M. *Biochem. J.* **1992**, *283*, 883-888.
9. Merkler, D. J.; Merkler, K. A.; Stern, W.; Fleming, F. F. *Arch. Biochem. Biophys.* **1996**, *330*, 430-434.
10. Mehta, N. M.; Gilligan, J. P.; Jones, B. N.; Bertelsen, A. H.; Roos, B. A.; Birnbaum, R. S. *Arch. Biochem. Biophys.* **1988**, *261*, 44-54.
11. a) Zabriskie, T. M.; Klinge, M.; Szymanski, C. M.; Cheng, H.; Vederas, J. C. *Arch. Insect Biochem. Physiol.* **1994**, *26*, 27-48. b) Kolhekar, A. S.; Roberts, M. S.; Jiang, N.; Johnson, R. C.; Mains, R. E.; Eipper, B. A.; Taghert, P. H. *J. Neurosci.* **1997**, *17*, 1363-1376.
12. Southan, C.; Kruse, L. I. *FEBS Lett.*, **1989**, *255*, 116-120.
13. Blackburn, N. J.; Hasnain, S. S.; Pettingill, T. M.; Strange, R. W. *J. Biol. Chem.* **1991**, *266*, 23120-23127.
14. Tian, G; Berry J. A.; Klinman, J. P. *Biochemistry* **1994**, *33*, 226-234.

15. Merkler, D. J.; Kulathila, R.; Consalvo, A.; Young, S. D.; Ash, D. E. *Biochemistry* **1992**, *31*, 7282-7288.

16. Merkler, D. J.; Kulathila, R.; Ash, D. E. *Arch. Biochem. Biophys.* **1995**, *317*, 93-102.

17. Zabriskie, T. M.; Cheng, H.; Vederas J. C. *J. Am. Chem. Soc.* **1992**, *114*, 2270-2272.

18. Klinge, M.; Zabriskie, T. M.; Cheng, H.; Vederas, J. C. *J. Chem. Soc., Chem. Commun.* **1994**, 1378-1380.

19. Erion, M. D.; Tan, J.; Wong, M.; Jeng, A. Y. *J. Med. Chem.* **1994**, *37*, 4430-4437.

20. Bertelsen, A. H.; Beaudry, G. A.; Galella, E. A.; Jones, B. N.; Ray, M. L.; Mehta, N. M. *Arch. Biochem. Biophys.* **1990**, *279*, 87-96.

21. Casara, P.; Ganzhorn, C.; Chanal, P. M. C.; Danzin, C. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 393-396.

22. Rhodes, C. H.; Honsinger, C. *N. Y. Acad. Sci.* **1994**, 663-666.

23. a) Pinner, A.; Spilker, A. *Ber.* **1889**, *22*, 685-698. b) Hines, J. W. Jr.; Breitholle, E. G.; Sato, M.; Stammer, C. H. *J. Org. Chem.* **1976**, *41*, 1466-1469. c) Greenlee, W. J. *J. Org. Chem.* **1984**, *49*, 2632-2634.

24. Sakota, N.; Okita, K.; Matsui, Y. *Bull. Chem. Soc. Jpn.* **1970**, *43*, 1138-1141.

25. Baldwin, J. E.; Moloney, M. G.; North, M. *Tetrahedron* **1989**, *45*, 6319-6330.

26. Itaya, T.; Iida, T.; Shimizu, S.; Mizutani, A.; Morisue, M.; Sugimoto, Y.; Tachinake, M. *Chem. Pharm. Bull.* **1993**, *41*, 252-261.

27. Mulzer, J.; Pointner, A.; Chucholowski, A.; Brüntrup, G. *J. Chem. Soc., Chem. Commun.* **1979**, 52-54.

28. The synthesis of the *N*-acetylstyrylglycines and the tripeptides containing D-styryl glycine and L-styrylglycine and the biological testing was completed by Dr. Mark D. Andrews, a post-doctoral fellow in the group of Dr. John C. Vederas.

29. Aboderin, A. A.; Delpierre, G. R.; Fruton, J. S. *J. Am. Chem. Soc.* **1965**, *87*, 5469-5472.

30. Felix, A. M.; Wang, C. T.; Fournier, A. *Int. J. Peptide Protein Res.* **1988**, *31*, 86-97.

31. Pottorf, R.S.; Szeto, P. In *Encyclopedia of Reagents for Organic Synthesis;* Paquette, L. A. Ed.; John Wiley & Sons, Toronto, 1995; Vol. 4, pp 2430-2431.

32. Merkler, D. J.; Young, S. D. *Arch. Biochem. Biophys.* **1991**, *289*, 192-196.

33. Ploem, J. S.; Tanke, H. J. *Introduction to Fluorescence Microscopy* ; Oxford University Press, Toronto, 1987.

34. a) Stryer, L. *Biochemistry 3rd ed.;* W. H. Freeman & Co., New York, **1988**; pp 62-64. b) Stryer, L. *Biochemistry 3rd ed.;* W. H. Freeman & Co., New York, **1988**; pp 440-441.

35. Lethbridge, A.; Norman, R. O. C.; Thomas, C. B. *J. Chem. Soc., Perkin Trans. 1* **1973**, 35-38.

36. House, H. O.; Jones, V. K.; Frank, G. A. *J. Org. Chem.* **1964**, *29*, 3327-3333.

37. Laloncette, J. M.; Frêche, A.; Brindle, J. R.; Laliberté, M. *Synthesis* **1972**, 526-532.

38. Bellamy, F. D.; Oce, K. *Tetrahedron Lett.* **1984**, *25*, 839-842.

39. Tarbell, D. S.; Insalaco, J. A. *Proc. Natl. Acad. Sci. USA* **1972**, *69*, 730.

40. Shiori, T.; Ninomiya, K.; Yamada, S. *J. Am. Chem. Soc.* **1972**, *94*, 2597-2599.

41. Curtius, T. *Ber.* **1890**, *23*, 3023-3025.

42. Belleau, B.; Malek, G. *J. Am. Chem. Soc.* **1968**, *90*, 1651-1652.

43. Lott, R. S.; Chauhan, V. S.; Stammer, C. H. *J. Chem. Soc., Chem. Commun.* **1979**, 495-497.

44. Voland, A.; Markussen, J. *Int. J. Pept. Protein Res.* **1974**, *6*, 79-86.

45. Airhart, J.; Sibiga, S.; Sanders, H.; Khairallah, E. A. *Anal. Biochemistry* **1973**, *53*, 132-140.

46. Carpino, L. A.; Sadat-Aalaee, D.; Chao, H. G.; Deselms, R. H. *J. Am. Chem. Soc.* **1990**, *112*, 9651-9652.

47. Corey, E. J.; Székely, I.; Shiner, C. S. *Tetrahedron Lett.* **1977**, 3529-3532.

48. Hecht, S. M.; Kozarich, J. W. *Tetrahedron Lett.* **1973**, 1397-1400.

49. Deutscher, M. P. *Meth. Enzymol.* **1990**, *182*, 609.

50. a) Hofmann, K.; Titus, G.; Montibeller, J. A.; Finn, F. M. *Biochemistry* **1982**, *21*, 978-984. b) Funakoshi, S.; Fukuda, H.; Fujii, N. *J. Chromatogr.* **1993**, *638*, 21.

51. a) Stryer, L. *Biochemistry 3rd ed.;* W. H. Freeman & Co., New York, **1988**; pp 50-55. b) Monks S. A.; Gould, A. R.; Lumley, P. E.; Kemu, R.; Goss, N. H.; Norton R. S. *Biochimica et Biophysica Acta* **1994**, *1207*, 93-101.

52. The synthesis and testing of the the 5 carbon chain biotinylated phenylbutenoic acid and the ^{13}C labelled biotinylated phenylbutenoic acid analogue from the labelled methyl 4-nitro-4-phenyl-3-butenoic acid was completed by Dr. Mark Andrews.

53. Nielsen, A. T.; Houlihan, W. J. *Org. React.* **1968**, *16*, 1-438.

54. Felix, M.; Myrboh, B. *Tetrahedron Lett.* **1990**, *31*, 3757-3758.

55. Karabatsos, G. J.; Bushman, D. W. *Tetrahedron* **1975**, *31*, 1471-1475.

56. Greene, T. W.; Wuts, P. G. M. *Protective Groups in Organic Synthesis 2nd ed;* John Wiley & Sons, Inc., Toronto, 1991; pp 232-234.

57. Bryan, D. B.; Hall, R. F.; Holden, K. G.; Huffman, W. F.; Gleason, J. G. *J. Am. Chem. Soc.* **1977**, *99*, 2353-2355.

58. Corey, H. S.; McCormick, J. R. D.; Swensen, W.Q. *J. Am. Chem. Soc.* **1964**, *86*, 1884-1885.

59. Perrin, D. D.; Armarego, W. L.; Perrin, D. R., *Purification of Laboratory Chemicals, 2nd ed.;* Permagon: New York, 1980.

60. Watson, S. C.; Eastham, J. F. *J. Organometal. Chem.* **1967**, *9*, 165-168.

61. Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923-2925.

62. Cheng, H. *Mechanism and Inhibition of Peptide Amidation*, Ph.D. thesis, University of Alberta, 1992, 131.

63. Hartung, W. H.; Siminoff, R. *Org. React.* **1953**, *VII*, 263-326.

64. Cheng, H. *Mechanism and Inhibition of Peptide Amidation*, Ph. D. thesis, University of Alberta, 1992, 130-131.

65. Cheng, H. *Mechanism and Inhibition of Peptide Amidation*, thesis, University of Alberta, 1992, 133.

66. Creary, X. *Org. Synth.* **1985**, *64*, 207-215.

67. Hosangadi, B.; Dave, R. H. *Tetrahedron, Lett.* **1996**, *37*, 6375-6378.

68. Buonora, P. T.; Rosauer, K. G.; Longun, D. *Tetrahedron Lett.* **1995**, *36*, 4009-4112.

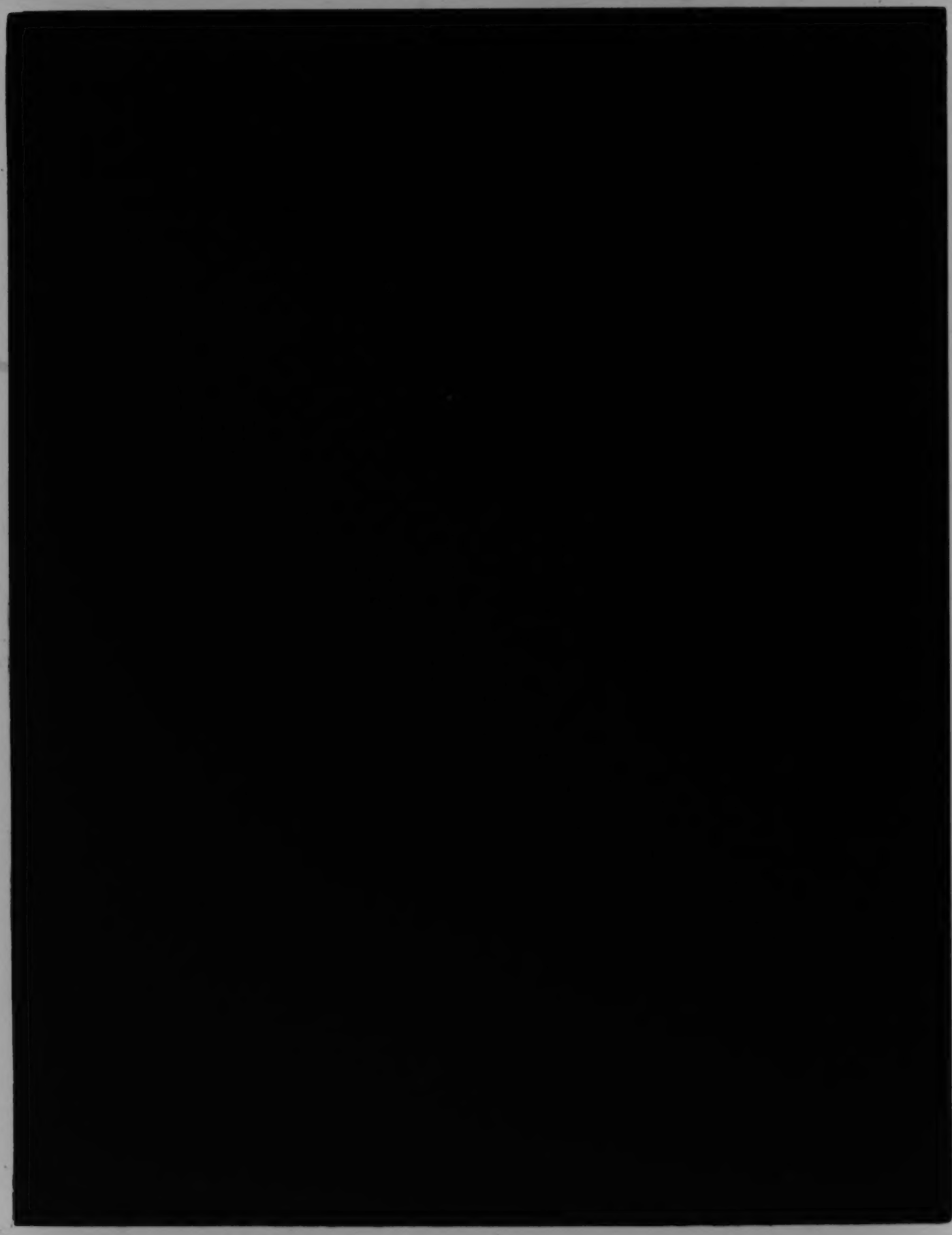
69. Zhang, Z.; Pan, Y.; Hu, H.; Kao, T. *Synthesis* **1991**, 539-542.

70. Thibonnet, J.; Abarbri, M.; Parrain, J. L.; Duchene, A. *Tetrahedron Lett.* **1996**, *37*, 7507-7510.

71. Ozaki, F.; Matsudura, M.; Kabasawa, Y.; Ishibashi, K.; Ikemori, M.; Hamano, S.; Minami, N. *Chem. Pharm. Bull.* **1992**, *40*, 2735-2740.

72. Andrews, M. D.; O'Callaghan, K. A.; Vederas, J. C. *Tetrahedron* **1997**, *53*, 8295-8306.

73. Jones, B. N.; Tamburini, P. P.; Consalvo, A. P.; Yong, S. D.; Lovato, S. J.; Gilligan, J. P.; Jeng, A. Y.; Wennogle, L. P. *Anal. Biochem.* **1988**, *168*, 272-279.

74. Bradford, M. M. *Anal. Biochem.* **1976**, 72, 248-254.

75. Villa, M. J.; Warren, S. *J. Chem. Soc., Perkin Trans. 1* **1994**, 1569-1572.